# THE COMPARATIVE ANATOMY AND PHYLOGENY OF THE BETULACEAE

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## Introduction

The anatomical method, especially in comparative studies of wood anatomy, is playing an ever increasing role in the attempt to arrive at a more natural system of angiosperm classification. The phylogenetic method in wood anatomy has produced, within recent decades, a series of anatomical dicta, or working principles, upon which comparative studies of wood anatomy are based. These dicta originated, for the most part, with the investigations of BAILEY and TUPPER (9), who measured the lengths of tracheary elements (tracheids, fibers, and vessels) in a wide range of vascular plants. They showed that, as evolution proceeds from lower to higher groups, these elements become progressively shorter. Further, specialization of the tracheary elements coincides with their evolutionary decrease in length, and this specialization continues in the dicotyledons. For example, as vessel members become shorter, they pass from the scalariform-perforated type to the simple, the slope of their end walls decreases, they become more nearly circular in cross-sectional outline, and their pitting passes from scalariform through a series of intermediate stages to alternate.

On the basis of the BAILEY and TUPPER investigation and others, the salient trends of anatomical specialization in the secondary wood of dicotyledons have been worked out by a number of investigators (4–6, 8, 11–13, 21, 26, 29–31, 34, 42–45, 47, 48, 70). The dicta thus obtained are adequately listed and explained in papers by TIPPO (74, 76) and MOSELEY (52).

Using the dicta as a tool, the relative phylogenetical development of the woods of a group may be determined. Then data can be drawn from other fields of botanical investigation, such as floral and inflorescence anatomy, nodal anatomy, pollen morphology, cytology, embryology, paleobotany, external morphology, etc. If all this information can be harmonized and correlated, a more nearly accurate picture of the phylogenetic position of the group in question can be obtained. As TIPPO (76) has indicated, this method is relatively free of bias, since it has been worked out by means independent of any preconceived system of angiosperm phylogeny.

The Betulaceae—the subject of the present investigation—are members of a group of plants, the "Amentiferae," whose phyletic position has long been debated. Studies of floral and inflorescence anatomy (1, 2) have indicated specialization within the family and afford evidence in favor of reduction. To supplement these studies, this comparative study of the secondary xylem has been undertaken to determine the degrees of specialization both within the family and of the family as a whole.

In this study the comparisons of secondary xylem have been at the level of the genus rather than that of the species

for several reasons, particularly: (*a*) sufficient variation does not exist among the woods of the species to be of morphological and statistical importance and (*b*) too few specimens of some species were available for study to comply with the standards proposed by RENDLE and CLARKE (66) for characters of diagnostic value. Since this study has not been at the species level, scant attention has been paid to possible taxonomic synonymy among the labeled specimens or to varietal names that have been appended to some species.

The Betulaceae, as ordinarily considered, consist of six genera in two tribes: *Betula* (birch) and *Alnus* (alder) in the tribe Betuleae and *Carpinus* (hornbeam), *Corylus* (hazel), *Ostrya* (hop-hornbeam), and *Ostryopsis* in the tribe Coryleae. These two tribes have been raised to family rank by some taxonomists (REGEL [63], WARMING [78], and HUTCHINSON [41]). Most taxonomists, and the most recent monograph (81), however, consider the tribes as parts of one family, whether it is called the Corylaceae (BAILEY [10], FERNALD [28], REHDER [64]) or the Betulaceae (RENDLE [65]).

The Betulaceae are predominately a family of north temperate, alpine, and arctic trees and shrubs, extending northward as far as any present-day arborescent angiosperms. *Betula* is found throughout most of the United States and Canada, in Eurasia, and from the Arctic Circle to Japan, China, the Himalayas, and southern Europe. *Alnus* is a genus on all continents of the Northern Hemisphere, extending southward in Asia as far as Assam, and in the New World extending through Mexico and Central America, and in the Andes to Peru, Bolivia, and northern Argentina. *Carpinus* occurs in Europe and Asia, with 1 species in eastern North America. *Corylus* and *Ostrya* are found in Eurasia and the United States and Canada. *Ostryopsis* is restricted to northern and western China. Many of the native members of the family are important timbers of commerce, and several of the Asiatic species have been introduced as ornamentals.

Early, brief, generalized descriptions of the wood structure of the Betulaceae were made by HARTIG (37) and SANIO (68) in the middle of the last century. There was, however, apparently no systematic approach to the study of the wood anatomy of this family until 1876. In that year MOELLER (51) presented a concise description of each of the principal genera, recognizing such things as spiral thickenings in the vessels of *Carpinus* and *Corylus*, the occurrence of the various types of vessel perforations in the different genera, and the occurrence of terminal wood parenchyma in *Betula* and *Alnus*. He also observed that the large rays in *Carpinus*, visible to the naked eye, were resolved under the microscope into numerous closely spaced narrow rays separated by one to several rows of fibers. HOULBERT (40) gave a cursory account of the wood anatomy of the Betulaceae but included the American species of *Fagus* with them. He has recorded certain general features of the wood anatomy, such as the pitting of the vessel walls, the width of the rays, and the occurrence of "false rays" (aggregate rays); he also described a fine spiral band in the vessels of some species of *Betula* and *Alnus*, which probably was a misinterpretation of the confluent apertures of the pits. BOUBIER (17) studied the leaf and wood anatomy of the Betulaceae. In *Corylus* he observed scalariform perforations in the vessel elements in the youngest wood, near the pith, and simple perforations in older wood; from this he concluded that the simple perforation replaced the scalariform in evolution and

that the scalariform was an atavistic retention. On the basis of available anatomical evidence, he concluded that the Betuleae are more primitive than the Coryleae. SOLEREDER's account (**71**), although based mainly on young wood obtained from herbarium specimens, differs in no essential details from the findings of this investigation, nor do the descriptions of METCALFE and CHALK (**50**). TIPPO's (**74**) descriptions also agree with those of the writer.

### Material and methods

Slides of 111 specimens purporting to represent 79 species (of about 105 species in the family) were examined. Some of these slides were furnished by Professor E. C. ABBE of the University of Minnesota, and most of the remainder are from the wood collection of the Biological Laboratories, Harvard University. These are listed below, with the following letter designations indicating the source of each specimen: A—slides from the collection of E. C. ABBE; RA—the collection of RAUP and ABBE; Y—slides made from the wood collection of the Yale School of Forestry, Yale University, and furnished by ABBE; H—the Harvard collection; I—a specimen from the herbarium of the University of Illinois. Also indicated is the relative age of the specimen, i.e., whether young wood or mature wood.[1] The classification of the family employed is that of WINKLER (**81**). REHDER (**64**) has been followed in the sectional listing of the species.

TRIBE BETULEAE

*Betula*

Section Eubetula

Subsection Nanae

*B. jackii* Schneid., H20312, young wood

*B. michauxii* Spach, A911, young wood

*B. nana* L., H19532, young wood

*B. pumila* L., A910, mature wood; Y11907, young wood

*B. sandbergii* Brit., H19537, young wood

Subsection Costatae

*B. bhojpattra* Wall., H20323, young wood

*B. chinensis* Maxim., Y16870, mature wood; Y22998, mature wood

*B. costata* Trautv., Y19956, mature wood; H7698, mature wood

*B. ermani* Cham., H19628, mature wood

*B. ermani* var. *communis* Koidz., H7701, mature wood

*B. ermani* var. *genuina* Winkl., H7702, mature wood

*B. grossa* Sieb. and Zucc., Y9314, mature wood; H7703, mature wood; H7704, mature wood

*B. lenta* L., H7708, mature wood; H23621, mature wood

*B. lutea* Michx., H7711, mature wood

*B. nigra* L., H7718, mature wood; H9843, mature wood

*B. schmidtii* Regel, Y13860, mature wood; H7731, mature wood

*B. ulmifolia* Sieb. and Zucc., H7732, mature wood

*B. utilis* D. Don, H7734, young wood

Subsection Albae

*B. alba* var. *verrucosa* (Ehrh.) Wall., H19524, young wood

*B. caerulea* Blanch., H20320, young wood

*B. caerulea-grandis* Blanch., H19525, young wood

*B. carpatica* Waldst. and Kit., H19526, mature wood

*B. davurica* Pall., Y9364, mature wood; H7700, mature wood

*B. fontinalis* Sarg., Y20583, mature wood; H19529, mature wood

*B. japonica* Sieb., Y9347, mature wood; H7705, mature wood; Y13859, mature wood

*B. japonica* var. *mandshurica* (Regel) Winkl., H7707, mature wood

*B. obscura* Kot., H19533, young wood

*B. occidentalis* Hook., H7719, mature wood

*B. odorata* Bechst., H19534, mature wood

*B. oycoviensis* Bess., H19535, young wood

[1] The term "young wood" refers to wood from a region near the pith; "mature wood" is wood relatively far from the pith.

*B. papyrifera* Marsh., Y11500, mature wood; H9846, mature wood
*B. populifolia* Marsh., Y11501, mature wood; H7724, mature wood; H9849, mature wood
*B. pubescens* Ehrh., H19536, mature wood

Section Betulaster
Subsection Acuminatae
*B. cylindrostachys* Lindl., H7699, mature wood
*B. luminifera* Winkl., H23911, mature wood
*B. maximowicziana* Regel, H7717, mature wood; H19530, mature wood

*Alnus*
Section Alnobetula
*A. alnobetula* C. Koch, H3321J, young wood
*A. crispa* (Ait.) Pursh, Y11904, young wood
*A. firma* var. *hirtella* Franch. and Sav., H11516, young wood
*A. maximowicziana* Callier, H19519, mature wood
*A. viridis* DC., H19523, young wood
Section Gymnothyrsus
*A. acuminata* HBK., H19514, mature wood
*A. cordata* (Loisel.) Desf., H7673, mature wood
*A. ferruginea* HBK., Y12455, young wood
*A. formosana* Makino, Y6797, mature wood; H19517, mature wood
*A. hirsuta* (Spach) Rupr., H7678, mature wood; H7676, mature wood
*A. hirsuta* var. *sibirica* (Spach) Schneid., H3585, mature wood
*A. incana* (L.) Moench, Y11192, mature wood; H9823, mature wood; Y11905, young wood; H9824, mature wood
*A. japonica* (Thunb.) Steud., H9828, young wood
*A. jorullensis* HBK., H19518, young wood
*A. maritima* (Marsh.) Nutt., H9830, mature wood
*A. mirbelli* Spach, H19520, young wood
*A. oblongifolia* Torr., Y14857, mature wood; H9833, mature wood
*A. oregona* Nutt., H9834, mature wood
*A. rhombifolia* Nutt., H7690, mature wood; H9836, mature wood; Y20579 mature wood
*A. rubra* Bong., H7688, young wood; Y20580, mature wood
*A. rugosa* (Du Roi) Spreng., Y11492, young wood
*A. serrulata* (Ait.) Willd., H7691, young wood
*A. tinctoria* Sarg., H3284, mature wood
*A. tinctoria* var. *glabra* Callier, H19521, mature wood; H3624, mature wood
*A. trabeculosa* Hand., H19522, young wood
Section Cremastogyne
*A. cremastogyne* Burk., H19515, mature wood

Tribe Coryleae
*Ostryopsis*
*O. davidiana* Dcne., H18075, young wood; I1125, young wood
*Ostrya*
*O. carpinifolia* Scop., Y7795, mature wood
*O. japonica* Sarg., H7755, mature wood
*O. liana* Hu, H9875, mature wood
*O. virginiana* (Mill.) K. Koch, H7757, mature wood; Y11505, mature wood
*Carpinus*
Section Distegocarpus
*C. carpinoides* Makino, H7747, mature wood
*C. cordata* Bl., H7746, mature wood; Y13809, mature wood
*C. erosa* Bl., Y23071, mature wood
*C. japonica* Bl., H9859, mature wood; H15141, mature wood
Section Eucarpinus
*C. betulus* L., Y16507, mature wood
*C. caroliniana* Walt., H7745, young wood; H9857, mature wood
*C. laxiflora* (Sieb. and Zucc.) Bl., Y14527, mature wood; H7750, mature wood
*C. orientalis* Mill., Y16209, mature wood
*C. tschonoskii* Maxim., Y14528, mature wood; H7751, mature wood
*C. turczaninowii* Hance, Y23011, young wood
*C. yedoensis* Maxim., H9861, mature wood; H7752, mature wood

*Corylus*

*C. americana* Marsh., Y11881, young wood
*C. avellana* L., Y19703, mature wood
*C. chinensis* Franch., H20873, young wood
*C. colurna* L., Y16210, mature wood
*C. heterophylla* var. *sutchuensis* Franch., H9867, young wood
*C. rostrata* Ait., H9869, mature wood; H9870, mature wood

The terms used in the anatomical descriptions are those recommended by the Committee on Nomenclature of the International Association of Wood Anatomists (**23**). The diagnostic characters largely follow TIPPO's list (**75**), with some reference to other lists (**61, 62**).

The classification of the imperforate tracheary elements as tracheids and fiber-tracheids conforms to BAILEY's (**5**) definitions. CHATTAWAY's (**20**) classification is used in describing the relative thickness of the walls of the fibrous elements, with reference to the radial walls, as follows:

| | |
|---|---|
| Very thin | Lumen much greater than thickness of walls |
| Thin | Lumen greater than thickness of walls |
| Thick | Lumen much less than thickness of walls |
| Very thick | Lumen almost completely closed |

Tangential diameters of 25–50 vessels were measured in transverse sections. The size classes (in $\mu$) followed are those of CHALK (**18**):

| | |
|---|---|
| Extremely small | Up to 25 $\mu$ |
| Very small | 25– 50 |
| Moderately small | 50–100 |
| Medium sized | 100–200 |
| Moderately large | 200–300 |
| Very large | 300–400 |
| Extremely large | Over 400 |

Twenty-five to fifty counts were made of the number of vessels per square millimeter, except in a few of the young-wood specimens where the number of vesssels was so great and their size so small that accurate counts could not be made under low magnification. In these instances an estimate was made of the range in vessel number, which in each case fell within the very numerous class. CHATTAWAY's (**20**) number classes are followed:

| | |
|---|---|
| Up to 2 | Very few |
| 2–5 | Few |
| 5–10 | Moderately few |
| 10–20 | Moderately numerous |
| 20–40 | Numerous |
| Over 40 | Very numerous |

For length of vessel elements (in $\mu$) the classification proposed by the Committee on the Standardization of Terms of Cell Size of the International Association of Wood Anatomists (**24**) is used:

| | |
|---|---|
| Extremely short | Less than 175 $\mu$ |
| Very short | 175–250 $\mu$ |
| Moderately short | 250–350 $\mu$ |
| Medium sized | 350–800 $\mu$ |
| Moderately long | 800–1100 $\mu$ |
| Very long | 1100–1900 $\mu$ |
| Extremely long | Over 1900 $\mu$ |

Measurements are of total body length, in so far as this was possible to determine by examining sections. Total body length has been shown by CHALK and CHATTAWAY (**19**) to represent most nearly the length of the fusiform initial from which the vessel element was derived. In sections, however, the tails or ligules of the vessel elements are often elusive, so these measurements have not been subjected to statistical analysis; furthermore, too few measurements were made to permit significant statistical study. Also, many specimens examined were of young wood, since many of the Betulaceae never attain more than a shrubby habit. BAILEY and TUPPER (**9**) and others have shown that the vessel element is short in young wood and gradually increases until a relatively stable length is attained. Thus,

comparisons should be made among specimens of the same relative age. These measurements are, therefore, only indicative of trends of specialization and are not to be considered diagnostic per se.

Measurements of thickness of the vessel walls of the Betulaceae show a range from 3 to 6 $\mu$; they are all classified as thin walled.

Scalariform perforation plates have been classified by FROST (30); those with 5 or fewer bars are placed in the "few" class, with 5–15 in the "intermediate" class, and over 15 in the "many" class. Plates with perforations less than 6 $\mu$ from bar to bar are classified as "narrow"; those with perforations more than 6 $\mu$ wide are "wide."

Ray classification follows that given by KRIBS (47). Width and height of rays, although variable from young to old wood in the same individual, and thus of little phylogenetic significance, have been grouped according to RECORD and CHATTAWAY (62): for ray width—1–2 cells, 3–4 cells, 5–10 cells, 10 or more cells; for ray height—fewer than 25 cells, 25–50 cells, 50–100 cells, and over 100 cells.

The types of distribution of xylem parenchyma follow, in part, the listing of BAILEY and HOWARD (7). These types are:

| Apotracheal | Paratracheal |
|---|---|
| Diffuse | Scanty |
| Metatracheal | Abundant |
| Terminal | Vasicentric |
| Initial | Aliform |
| | Confluent |

Some of these terms have been defined by the Committee on Nomenclature of the International Association of Wood Anatomists and later clarified by KRIBS (47), CHOWDHURY (22), and BAILEY and HOWARD (7). Descriptions of the abundance of the parenchyma have been somewhat arbitrary. A specimen which was considered to have abundant parenchyma was used as a standard, and comparisons of other species were made with it.

## Anatomical description of the genera

### BETULA

Thirty-three species of *Betula* in the four subsections of the genus were studied.

All the species of *Betula* have well-defined growth rings except *B. cylindrostachys*, a species from the Himalayas. PEARSON and BROWN (59) stated that the growth rings in this species are indistinctly delimited by two- to four-seriate bands of metatracheal parenchyma, but in the specimen examined the metatracheal parenchyma gave no clear evidence of growth rings. In the majority of species the growth rings range in width from about 60 $\mu$ in some shrubs to 4–5 mm. in mature trees. The width of the growth rings, however, is largely determined by external environmental conditions, and thus no diagnostic significance can be attached to this variation.

FIBROUS ELEMENTS.—Of these elements, only tracheids and fiber-tracheids occur in *Betula*. These are apparently scattered indiscriminately throughout the growth ring in no definite arrangement. Minute bordered pits occur, for the most part, on the radial walls; on the fiber-tracheids the pits have long, slitlike crossed apertures. The number of pits on each fiber varies, but, in general, fibers from young wood have more pits than fibers from more mature wood. Gelatinous fibers (fiber-tracheids in *Betula*) occur in several species, especially in young wood (fig. 7). There are no septate fibers, but local gum deposits in the fibers of some species give the appearance of septation in longitudinal sections. The

occurrence of tracheids and fiber-tracheids among the species is given in table 1. In the section Eubetula, tracheids predominate in the subsection Nanae, but fiber-tracheids also occur, except in *B. nana*. All the members of this subsection are shrubs and, except for certain hybrids, usually do not produce what may be called mature wood. Caution must be used in interpreting anatomical characters in the woods of these species. Thus, the predominance of tracheids in this subsection does not necessarily indicate primitiveness of the entire subsection, since it is well known that young-wood specimens in general exhibit primitive characters. In the subsection Costatae only fiber-tracheids occur in *B. chinensis*,

TABLE 1

CHARACTERISTICS* OF WOOD OF BETULA

| SPECIES | TRACHEIDS | FIBER-TRACHEIDS | WOOD PARENCHYMA | | | | | AGGREGATE RAYS | ANGULAR (A) OR CIRCULAR (C) VESSELS |
|---|---|---|---|---|---|---|---|---|---|
| | | | Terminal | Metatracheal | Diffuse | Scanty | Abundant | | |
| Section Eubetula: | | | | | | | | | |
| Subsection Nanae: | | | | | | | | | |
| *Betula jackii*† | + | s | + | + | | | + | | A |
| *B. michauxii*† | + | s | + | + | s | | + | + | A |
| *B. nana*† | + | | + | + | | | + | + | A |
| *B. pumila*† | + | s | + | + | s | | + | + | A |
| *B. sandbergii*† | + | s | + | + | s | | + | | A |
| Subsection Costatae: | | | | | | | | | |
| *B. bhojpattra*† | + | s | + | + | + | + | | | A |
| *B. chinensis* | | + | + | s | + | + | | | A |
| *B. costata* | + | s | + | | + | + | | | A–C |
| *B. ermani* | + | s | + | s | + | + | | | A–C |
| *B. grossa* | s | + | + | | + | + | | | A–C |
| *B. lenta* | s | + | + | s | + | + | | | A |
| *B. lutea* | | + | + | | + | + | | | A |
| *B. nigra* | | + | + | s | + | + | | | A–C |
| *B. schmidtii* | + | s | + | s | + | + | | | A |
| *B. ulmifolia* | + | s | + | s | + | + | | | A |
| *B. utilis*† | + | s | + | s | + | + | | | A |
| Subsection Albae: | | | | | | | | | |
| *B. alba* var. *verrucosa*† | + | | + | + | s | | + | | A |
| *B. caerulea*† | + | s | + | + | s | | + | | A |
| *B. caerulea-grandis*† | + | s | + | + | s | | + | | A–C |
| *B. carpatica* | + | s | + | + | s | | + | | A–C |
| *B. davurica* | s | + | + | + | s | | + | | A |
| *B. fontinalis* | + | s | + | + | s | | + | | A–C |
| *B. japonica* | s | + | + | + | s | | + | | A |
| *B. obscura*† | s | + | + | + | s | | + | | A |
| *B. occidentalis* | s | + | + | + | s | | + | | A |
| *B. odorata* | + | s | + | + | s | | + | | A |
| *B. oycoviensis*† | + | s | + | + | s | | + | | A |
| *B. papyrifera* | + | s | + | + | s | | + | | A |
| *B. populifolia* | + | s | + | + | s | | + | + | A |
| *B. pubescens* | s | + | + | + | s | | + | | A |
| Section Betulaster: | | | | | | | | | |
| Subsection Acuminatae: | | | | | | | | | |
| *B. cylindrostachys* | | + | | + | | | + | | A–C |
| *B. luminifera* | s | s | + | + | | + | | | A |
| *B. maximowicziana* | | + | + | + | | | + | | A–C |

* s = Present, but not predominant: + = characteristically present.

† = Young-wood specimen.

*B. lutea*, and *B. nigra;* otherwise the distribution of tracheids and fiber-tracheids is fairly even among the species of this subsection. In the subsection Albae only tracheids are found in *B. alba* var. *verrucosa;* but, again, this is represented by a young-wood specimen. Again, the distribution of tracheids and fiber-tracheids is fairly even throughout the subsection. In the section Betulaster, subsection Acuminatae, only fiber-tracheids are found in 2 species, and both fiber-tracheids and tracheids in 1 species.

The thickness of the radial wall of the fibers varies among the species from very thin to thick (figs. 3, 5, 6, 8, 10). At the boundary of the growth rings in most species there are two to four rows of fibers which are greatly flattened radially, so that the lumen is almost completely closed, and they are thus classified as very thick walled.

VESSELS.—All members of the Betulaceae have diffuse-porous wood. In *Betula* the vessels occur as solitary pores, some pore clusters, and mostly pore multiples, the latter having 2–10, mostly 2–5 vessels. In the late wood of the growth ring the pore multiples sometimes consist of numerous vessels greatly flattened radially, often indistinguishable from fibers.

A summary of vessel measurements is given in table 2. The range in number of vessels per square millimeter in the genus is from 7, in the subsection Costatae, to 684, in the subsection Nanae. The range in average number of vessels per square millimeter varies from 211 (very numerous) in the Nanae to 24 (numerous) in the Acuminatae. In the Nanae the vessel number of two hybrids, *B. jackii* and *B. sandbergii*, is intermediate between that of the natural species of the subsection and that of the subsection in which their second parent occurs (Costatae and Albae, respectively). Thus, if the figures for the two hybrids are excluded from the calculation of the mean vessel number of the subsection Nanae, the mean is raised to 268/sq mm. Both of these figures, however, are in the very numerous class. The average of means of all species of *Betula* is 81; excluding the young-wood specimens this figure becomes 42 (very numerous), a more nearly representative figure for the number of vessels per square millimeter in *Betula*.

Vessel diameters in the genus range from 8 $\mu$ in the subsection Nanae to

TABLE 2

VESSEL MEASUREMENTS IN BETULEAE

| GENUS AND SUBSECTION | NUMBER PER SQ. MM. | | | DIAMETER IN $\mu$ | | | LENGTH IN $\mu$ |
|---|---|---|---|---|---|---|---|
| | Range | Most frequent | Mean | Range | Most frequent | Mean | Mean |
| *Betula:* | | | | | | | |
| Nanae | 49–684 | 65–500 | 211 (268)* | 8–56 | 18– 50 | 30 | 430 (352)* |
| Costatae | 7–119 | 10–110 | 43 (34)† | 11–147 | 24–125 | 66 (71)† | 783 (820)† |
| Albae | 9–285 | 12–155 | 65 (55)† | 13–112 | 25–100 | 52 (57)† | 653 (704)† |
| Acuminatae | 13– 53 | 15– 40 | 24 | 36–168 | 60–130 | 90 | 775 |
| *Alnus* | 6–310 | 35–150 | 90 (74)† | 13– 90 | 25– 65 | 47 (52)† | 664 (716)† |

* Excluding hybrids.

† Means of mature woods only.

168 μ in the Acuminatae, while the mean vessel diameters range from 30 μ in the Nanae to 90 μ in the Acuminatae. The average of means of all species of *Betula* is 57 μ; excluding the young-wood specimens, this figure becomes 67 μ (moderately small). Mean length of vessel elements varies from 310 μ in *B. nana* to 1013 μ in *B. ulmifolia*. Mean lengths of vessel elements of the subsections of the genus are given in table 2. The average of the means of all species of *Betula* is 673 μ; excluding the young-wood specimens, the average is 761 μ (medium sized) for the genus.

The figures for vessel length are from measurements taken from sections and include, as far as possible, total body length of the vessel elements. For comparison, wood of 3 available species was macerated, and measurements of vessel-element length were made. In 2 of the species thus compared (*B. bhojpattra* and *B. jackii*) the mean length of vessel elements was 53 and 45 μ longer, respectively, in the macerated material than in sections, and in the third species (*B. caerulea*) the length was approximately the same in sections and macerated material. Thus, the figures given for vessel-element length are probably shorter than the actual total body length but are not so much shorter as to put the vessel elements in a different size category.

Intervascular pitting is alternate in all species of *Betula* (fig. 17), except in the young wood of *B. michauxii* and *B. nana*, where some opposite pitting occurs. The pits are minute (2–4 μ in diameter), closely spaced, circular or somewhat angular owing to crowding, and the pit orifice is usually included within the border of the pit. In some vessel elements the pits are so numerous that, because of their contiguous, alternate arrangement and confluent apertures, they appear to form a spiral band within the vessel.

The vessels are angular in cross section in 24 species and angular to circular in 9 species (figs. 3, 5, 6, 8, 9, 10).

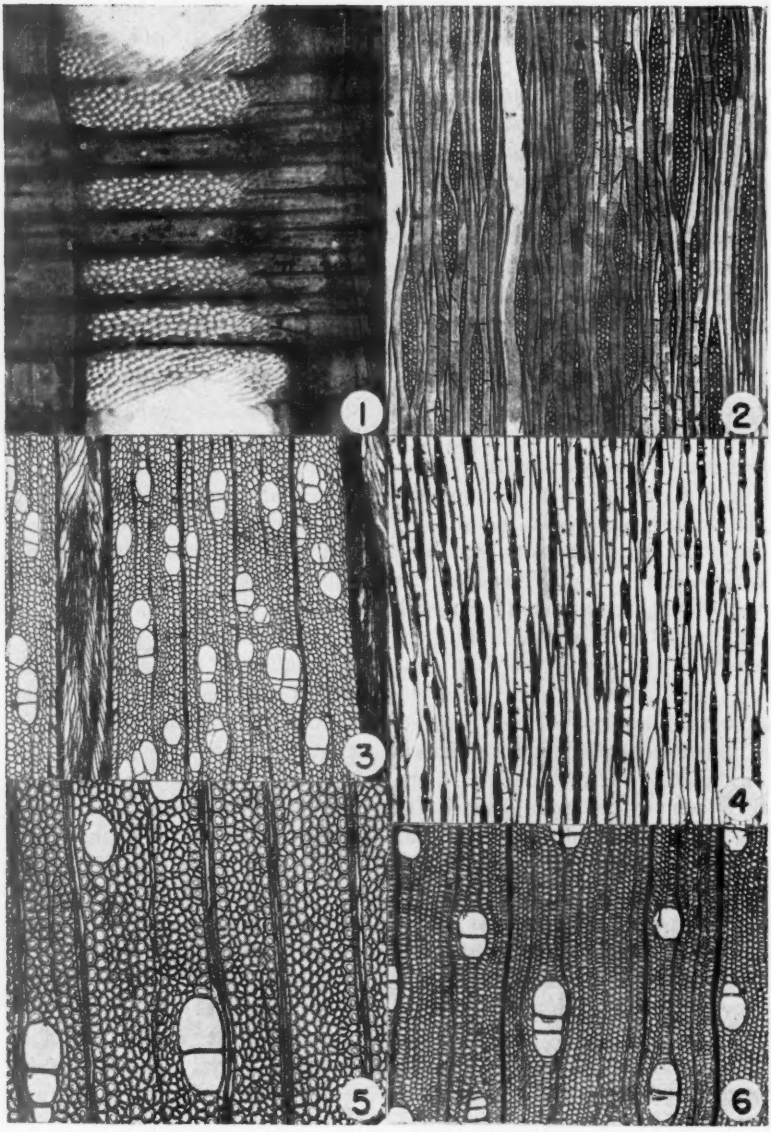
The perforation plates are exclusively scalariform; most of the species have nonbordered plates. In the section Eubetula, subsection Nanae, all species have many bars in the perforation plates. In the Costatae 5 species have many bars, 5 have intermediate to many bars, and 1 has few to intermediate bars. In the subsection Albae, 7 species have many bars, and 7 have intermediate to many bars. In the section Betulaster, subsection Acuminatae, 1 species has intermediate bars, and 2 species have few to intermediate bars. The width of the perforations (i.e., distance between bars) varies from 4 (narrow) to 13 μ (wide). In the section Eubetula, subsection Nanae, 4 species have narrow perforations, and 1 (*B. sandbergii*) has narrow to wide perforations. In the Costatae 1 species has narrow perforations, 7 have narrow to wide perforations, and 3 have wide perforations. In the Albae 6 species have narrow perforations, 7 have narrow to wide perforations, and 1 has wide perforations. In the section Betulaster, subsection Acuminatae, all species have wide perforations.

The angle of the end wall of the vessel element is 10°–45°, mostly 10°–35°.

Gum deposits occur in the vessels of 6 species. Tyloses are absent. The vessel wall is thin (3–6 μ) in all species.

Ray-vessel pitting is alternate, minute, and bordered (fig. 1).

The vascular rays are homogeneous I and are 1–5 cells wide, mostly 1–3 cells (figs. 2, 4, 11). Occasionally heterogeneous rays occur. In *B. michauxii* there is a tendency toward the elimination of multiseriate rays (figs. 4, 10). The height of the uniseriate rays is 1–25 cells, mostly




FIGS. 1–6.—Fig. 1, *Betula grossa:* radial section showing alternate ray-vessel pitting. ×450. Fig. 2, *B. japonica:* tangential section at boundary of growth ring, showing multiseriate rays and terminal wood parenchyma. ×65. Fig. 3, *B. populifolia:* cross section depicting aggregate rays, scanty, diffuse wood parenchyma, thin-walled tracheids, and angular vessels. ×50. Fig. 4, *B. michauxii:* tangential section showing predominance of uniseriate rays. ×80. Fig. 5, *B. chinensis:* cross section with angular vessels, thick-walled tracheids, and multiseriate rays. ×90. Fig. 6, *B. schmidtii:* cross section showing abundant metatracheal parenchyma in short tangential bands. ×70.

2–10 cells; that of the multiseriate rays is 4–67 cells, mostly 8–20. The number of rays per millimeter is 6–22 and averages 10. Aggregate rays are present in 5 species. They occur in the natural species of the subsection Nanae but are lacking in the two hybrids. They also are well developed in *B. populifolia* (fig. 3). Crystals occur in the ray cells of several species, as do gum deposits, especially at the boundary of the growth rings (fig. 12).

XYLEM PARENCHYMA.—The distribution of xylem parenchyma is given in table 1. All species, except *B cylindrostachys*, have terminal parenchyma in the form of a more or less distinct uniseriate row of cells (fig. 8). Metatracheal parenchyma in the form of uniseriate, meandering bands of 2–5 cells predominates in the subsections Nanae, Albae, and Acuminatae (figs. 9, 13). In the Costatae, metatracheal parenchyma occurs only sporadically and is abundant only in *B. bhojpattra*. Diffuse parenchyma occurs in almost all species of the Nanae, Albae, and Costatae but is absent in the section Betulaster, subsection Acuminatae. In the section Eubetula it predominates in the Costatae but occurs only to a limited extent in the Nanae and Albae. The comparative amount of parenchyma is scanty in the Costatae and abundant in the Nanae and Albae, except for *B. japonica*, where it is approximately intermediate in amount. In the Acuminatae, *B. luminifera* has scanty parenchyma, and *B. cylindrostachys* and *B. maximowicziana* have abundant parenchyma. *B. cylindrostachys* is unique among the Betulaceae in having two- to four-seriate bands of metatracheal parenchyma, which, PEARSON and BROWN (**59**) stated, indicate the boundary of growth rings. Sieve-pitting occurs between adjacent parenchyma cells.

Pith flecks (fig. 14) occur in 19 species.

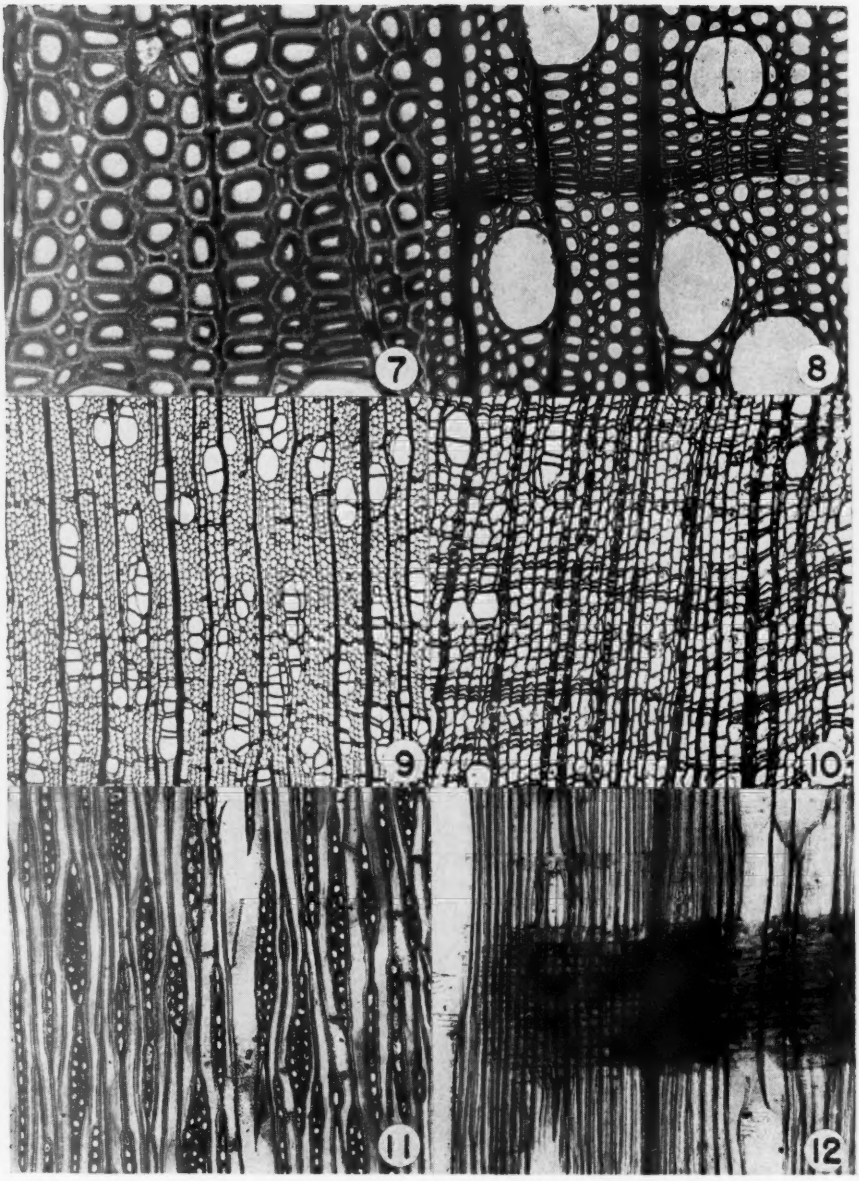
ALNUS

Twenty-four species of *Alnus* were studied. Only three of the four sections of the genus are represented; no specimens of the section Clethropsis were available, and in the section Cremastogyne only one specimen was studied.

Many of the species of *Alnus* are shrubs and are represented by young-wood specimens only. This somewhat obscures the trends of specialization within the genus, since in many cases young-wood specimens show anatomical characters different from mature-wood specimens of the same species. The occurrence of so many young woods is probably one reason why there appears to be less anatomical consistency among the sections of *Alnus* than among the sections of *Betula*. Consequently, the anatomical discussion of the genus *Alnus* will not be considered from the level of the section, except to indicate certain clear-cut distinctions. In many features of its wood anatomy *Alnus* is very similar to *Betula*.

Growth rings are present in all species except *A. acuminata*, a Central American species. They have approximately the same range in width as those of *Betula*. In those species with aggregate rays, the growth rings are wavy in outline.

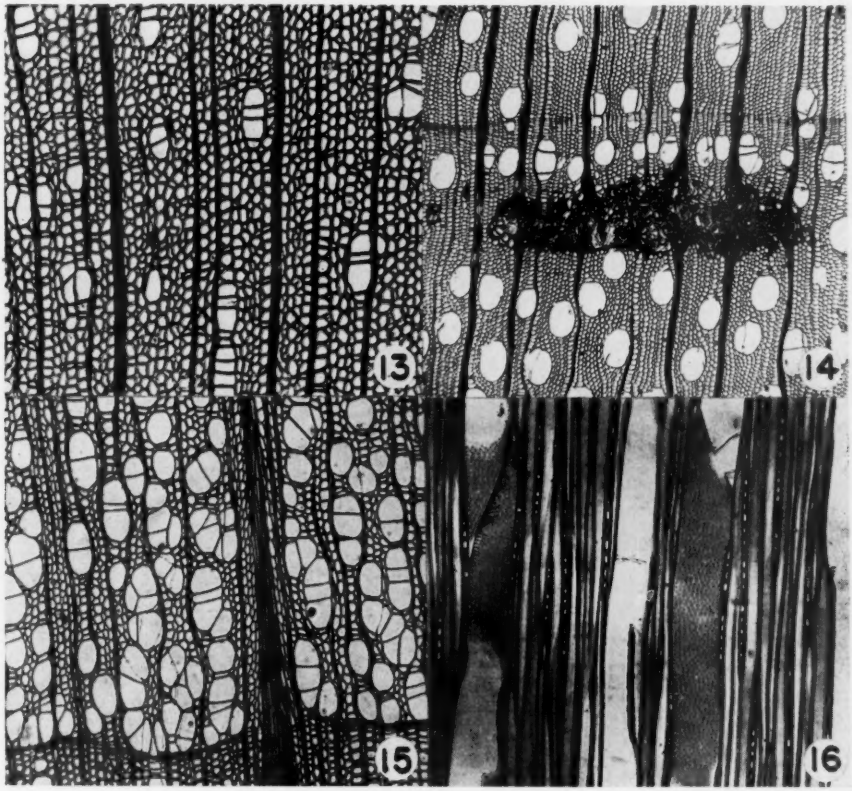
Tracheids and fiber-tracheids occur in *Alnus*. The pits on radial walls are much larger than those of the corresponding elements in *Betula;* thus, fibrous elements which are fiber-tracheids in *Alnus* would be tracheids in *Betula*. This situation arises because the intervascular pitting in *Alnus* is larger than that in *Betula*. Tracheids and fiber-tracheids occur in 15 species, and only fiber-tracheids in 9 species. Thickness of the radial wall varies from very thin to thick (figs. 21, 22, 23), except at the boundary of the growth rings, where the fibers are very




FIGS. 7–12.—Fig. 7, *Betula oycoviensis:* cross section with thick-walled gelatinous fibers. ×545. Fig. 8, *Betula* sp.: cross section showing terminal parenchyma, angular to round vessels, and thin to thick-walled tracheids and fiber-tracheids. ×150. Fig. 9, *B. alba* var. *verrucosa:* cross section to illustrate metatracheal parenchyma, angular vessels in long pore multiples, pore clusters, and solitary pores. ×70. Fig. 10, *B. michauxii:* cross section showing incipient aggregate ray. ×110. Fig. 11, *B. chinensis:* tangential section with generally low multiseriate rays. ×95. Fig. 12, *B. ermani* var. *genuina:* radial section showing gum deposits in parenchyma cells and ray cells at boundary of growth ring. ×85.

thick walled, as in *Betula*. Gelatinous fibers occur in some young-wood specimens. Septate fibers are absent, but local gum deposits in some fibers give the appearance of septation.

most frequent range 35–150/sq mm. The average of the means is given in table 2. In many species of *Alnus* the vessels are very crowded (fig. 15). *A. acuminata* is an exception, having moderately few,




FIGS. 13–16.—Fig. 13, *Betula pumila:* cross section showing abundant metatracheal parenchyma in short tangential bands. ×100. Fig. 14, *B. nigra:* cross section of pith fleck. ×35. Fig. 15, *Alnus rhombifolia:* cross section of aggregate ray. ×70. Fig. 16, *A. rubra:* tangential section showing opposite to alternate intervascular pitting. ×80.

*Alnus* is diffuse-porous; the vessels occur as solitary pores, pore clusters, and mostly pore multiples (figs. 15, 22, 23, 26). The latter have 2–12 vessels, mostly 2–8, and they are radially arranged.

The number of vessels per square millimeter ranges from 6 in *A. acuminata* to 310 in *A. crispa* (young wood), with the

widely spaced, angular to circular vessels (fig. 21).

Vessel diameter ranges from 13 μ in *A. viridis* to 90 μ in *A. oblongifolia*. The most frequent range is 25–65 μ.

The mean length of the vessel elements ranges from 376 μ in *A. crispa* (young wood) to 878 μ in *A. formosana*. The

average of means of all woods is 664 $\mu$ (medium-sized), of the mature woods 716 $\mu$, and of the young woods 575 $\mu$.

Intervascular pitting is transitional (some), opposite, and alternate (table 3). In the section Alnobetula transitional is some opposite pitting (fig. 24); alternate pitting is common (fig. 16). The pits are small, 4.5–6.5 $\mu$ in diameter.

The vessels are angular in cross section in 21 species and angular to round in 3 species.

TABLE 3

INTERVASCULAR PITTING, AGGREGATE RAYS, AND WOOD PARENCHYMA DISTRIBUTION IN ALNUS

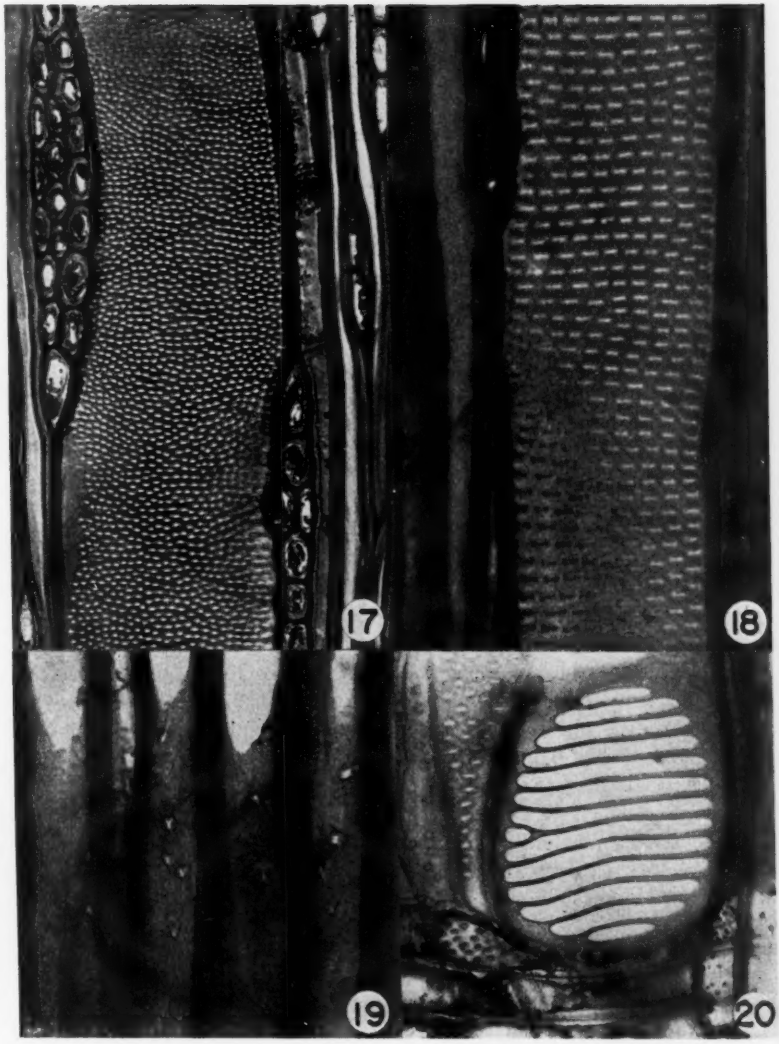
| Species | Intervascular pitting | | | Aggregate rays | Wood parenchyma | | | |
|---|---|---|---|---|---|---|---|---|
| | Transitional | Opposite | Alternate | | Diffuse | Metatracheal | Scanty | Abundant |
| Section Alnobetula: | | | | | | | | |
| *Alnus alnobetula** | s† | + | | − | + | + | | + |
| *A. crispa** | s | + | | − | + | + | | + |
| *A. firma* var. *hirtella** | | + | | − | + | + | | + |
| *A. maximowicziana* | | + | | − | + | + | + | |
| *A. viridis** | | + | | − | + | + | | + |
| Section Gymnothyrsus: | | | | | | | | |
| *A. acuminata* | | | + | ± | + | + | + | |
| *A. cordata* | s | s | s | + | + | + | + | |
| *A. ferruginea** | s | s | + | − | + | + | | + |
| *A. formosana* | | s | + | + | + | + | + | |
| *A. hirsuta* | | | + | ± | + | + | + | |
| *A. hirsuta* var. *sibirica* | s | s | + | + | + | + | + | |
| *A. incana* | | s | + | + | + | + | + | |
| *A. incana** | | s | + | + | + | + | | + |
| *A. japonica** | s | s | + | + | + | + | | + |
| *A. jorullensis** | s | s | | − | + | + | + | |
| *A. maritima* | | s | + | + | + | + | + | |
| *A. mirbelli** | | s | + | − | + | + | + | |
| *A. oblongifolia* | | s | + | − | + | | + | |
| *A. oregona* | | s | + | + | + | | + | |
| *A. rhombifolia* | | s | + | + | + | | + | |
| *A. rubra* | | s | + | + | + | + | + | |
| *A. rubra** | | s | + | − | + | + | | + |
| *A. rugosa** | | + | s | + | + | + | | + |
| *A. serrulata** | | | + | + | + | + | | + |
| *A. tinctoria* | | s | + | − | + | + | + | |
| *A. tinctoria* var. *glabra* | | s | + | + | + | + | + | |
| *A. trabeculosa* | | s | + | + | + | + | | + |
| Section Cremastogyne: | | | | | | | | |
| *A. cremastogyne* | | | + | + | + | + | + | |

* = Young-wood specimen.

† s = Present, but not predominant; + = characteristically present or predominant; − = lacking; ± = present, but poorly developed.

pitting occurs in the very young wood of *A. crispa;* opposite pitting predominates otherwise (fig. 18), and there is no alternate pitting. In the section Gymnothyrsus transitional pitting occurs in the young wood of several species, and there

*Alnus* has scalariform perforation plates, most of which are not bordered. The range in number of bars is 8–27. There are many bars in 13 species and intermediate to many bars in 11 species (fig. 20). The perforations are mostly




FIGS. 17–20.—Fig. 17, *Betula chinensis:* tangential section showing alternate intervascular pitting. ×690. Fig. 18, *Alnus crispa:* tangential section to illustrate opposite intervascular pitting. ×910. Fig. 19, *A. incana:* radial section to illustrate fiber-tracheids. ×610. Fig. 20, *A. rhombifolia:* radial section showing bordered scalariform perforation plate with intermediate number of bars. ×435.

from 3 to 8 $\mu$ wide. Eighteen species have narrow perforations, and 6 species have narrow to wide perforations.

The angle of the end wall ranges from 5° to 40°, mostly 10°–30°.

Gum deposits are found occasionally in the vessels of 1 species (*A. tinctoria* var. *glabra*) and in the ray parenchyma of 2 species (figs. 23, 24). Tyloses are absent.

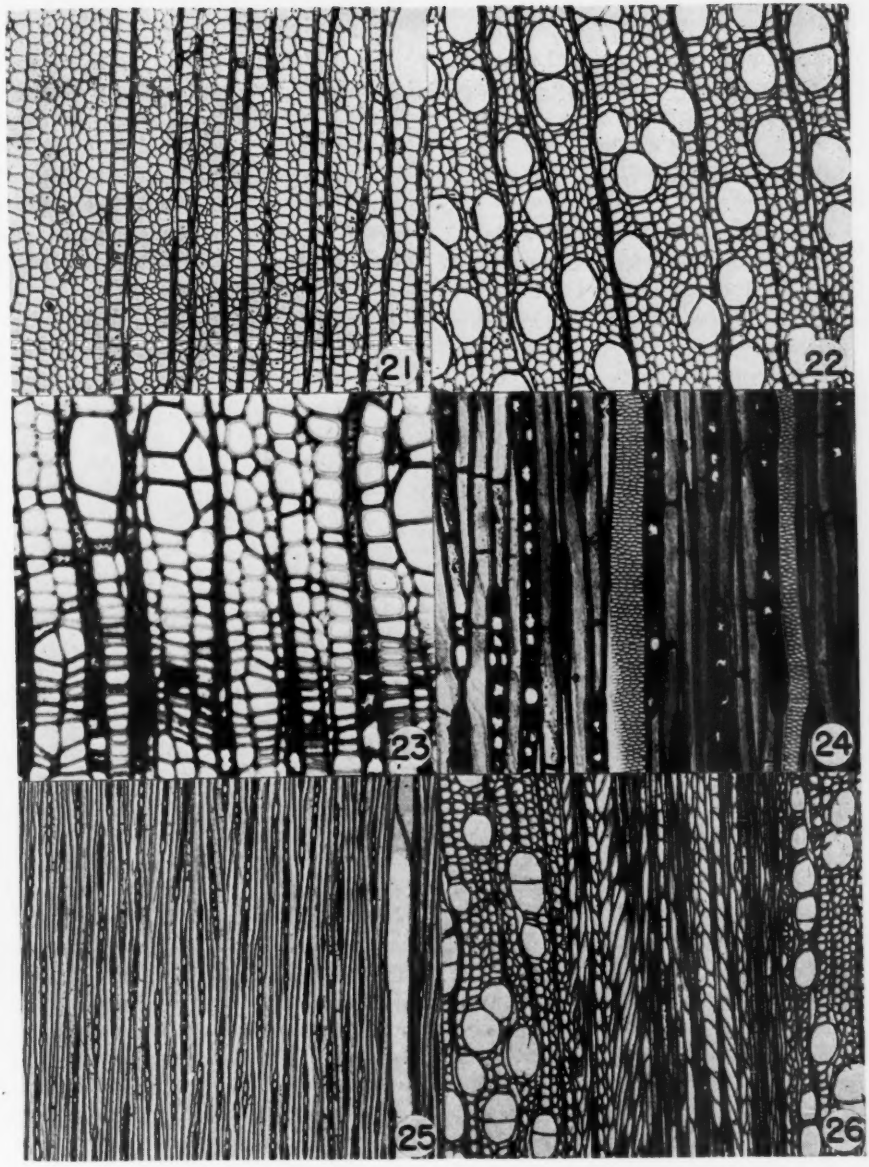
The vascular rays are homogeneous I. There is a marked tendency toward reduction of multiseriates, so that some species (*A. crispa*, fig. 25) appear to lack them completely. The rays are 1–3 cells wide but are mostly uniseriate. The uniseriate rays are 1–67 cells high, mostly 5–25 cells. The number of rays per millimeter is 8–25 and averages 13.5. Aggregate rays are found in 17 species (figs. 15, 26; table 3); they are poorly developed in *A. acuminata*. No aggregate rays are found in the section Alnobetula.

The types of xylem-parenchyma distribution are given in table 3. All species have diffuse parenchyma, and most have metatracheal parenchyma in the form of meandering, uniseriate bands of 2–5 cells. The relative abundance of the wood parenchyma apparently is determined by the age of the wood. In general, the more mature woods have scanty parenchyma, while the young woods have abundant parenchyma.

Pith flecks occur in 7 species.

The two genera, *Betula* and *Alnus*, comprise the tribe Betuleae, which may be characterized as follows: Fibrous elements consist of tracheids and fiber-tracheids, sometimes containing gum deposits, and with a radial wall varying from very thin to thick, and very thick at the boundary of the growth rings. Wood diffuse-porous, the vessels occurring as solitary pores, pore clusters, and pore multiples of 2–12, mostly 2–8 vessels. Vessels very numerous, moderately small, and of medium size. Intervascular pitting is rarely transitional, sometimes opposite, and mostly alternate, and is minute to small in size. Vessels are angular in cross section for the most part but tend to be round in some species. Perforation plates are exclusively scalariform, with mostly nonbordered bars. The number of bars ranges from few to many, mostly intermediate to many in *Betula* and many in *Alnus*. The width of the perforations is from narrow to wide, mostly narrow in *Alnus*. The angle of the end wall ranges from 5° to 45° and is mostly 10°–35°. Gum deposits occur in the vessels of some species; tyloses are absent. The vessel walls are thin in both genera. The vascular rays are homogeneous I. Width of the rays is 1–5 cells, mostly 1–3 cells in *Betula* and mostly uniseriate in *Alnus*. Height of uniseriate rays is 1–67 cells, mostly 2–25 cells; multiseriates are 4–67 cells high, mostly 8–20 cells. Aggregate rays occur in both genera but predominate in *Alnus;* in *Betula* they are best developed in the subsection Nanae, and in *Alnus* they are lacking in the section Alnobetula. Crystals occur in the ray parenchyma cells of some species of *Betula*, and gum deposits are frequent in the rays of both genera. Xylem parenchyma is diffuse, metatracheal, and terminal in *Betula*, and diffuse and metatracheal in *Alnus*. Abundance of the parenchyma varies among the species and sections of the genera. All parenchyma cells are lignified. Pith flecks are fairly common, occurring in at least 26 species.

The wood of *Alnus* may be anatomically distinguished from that of *Betula* by the opposite and alternate intervascular pits in the former, which are somewhat larger than those in *Betula* (2–4 $\mu$ in *Betula*, 4.5–6.5 $\mu$ in *Alnus*), and in addition by the tendency toward elimina-




FIGS. 21–26.—Fig. 21, *Alnus acuminata:* cross section with poorly defined aggregate ray and scanty wood parenchyma. ×80. Fig. 22, *A. cremastogyne:* cross section with numerous, angular, solitary vessels, pore clusters and short pore multiples, and uniseriate rays. ×95. Fig. 23, *A. mirbelli:* cross section to show gummy nature of parenchyma cells. ×220. Fig. 24, *A. jorullensis:* tangential section showing gum deposits in rays and opposite intervascular pitting. ×140. Fig. 25, *A. crispa:* tangential section with numerous, mostly uniseriate rays. ×90. Fig. 26, *A. rhombifolia:* cross section showing aggregate ray, numerous vessels in pore clusters, pore multiples, and solitary pores. ×65.

tion of multiseriate rays and possession of aggregate rays in most species of *Alnus*.

### Ostryopsis

One species of *Ostryopsis*, *O. davidiana*, was studied.

Growth rings are slightly wavy in outline because of the presence of aggregate rays. In the specimens examined the rings are approximately 1–1.5 mm. wide (fig. 44).

The fibrous elements consist entirely of fiber-tracheids. They are very thin to thin walled. The rows of very thick-walled fibers occurring at the boundary of the growth rings in the Betuleae are lacking in *Ostryopsis*.

Pore distribution is solitary, pore clusters, and pore multiples of two to many vessels (fig. 41). The vessels occur in flamelike groups. The vessels gradually decrease in diameter toward the boundary of the growth ring (fig. 44). This arrangement of the vessels is also found in *Corylus* but not in *Ostrya* and *Carpinus*. The vessels are angular and thin walled.

Range in vessel number per square millimeter is 157–229, with a mean of 175 (very numerous). Vessel diameters are 11–60 $\mu$, most frequently 20–40 $\mu$, and average 33 $\mu$ (very small). Length of vessel elements averages 351 $\mu$ (medium sized).

The intervascular pitting is alternate and composed of small to medium-sized, circular, crowded pits. The pits are 4.5–9 $\mu$ in diameter. Spiral thickenings are present in most of the vessels.

Perforation plates are scalariform with an intermediate number of bars. The perforations are wide and bordered. The angle of the end wall is 5°–25°.

Gum deposits and tyloses are absent.

Vascular rays are heterogeneous IIB, with a tendency toward homogeneity. They are mostly 1–2 cells wide and are 1–15 cells high, mostly 1–10 cells. Poorly developed aggregate rays are numerous (fig. 44). In cross section they appear to meander rather than to run in straight, radial rows; tangential sections show the typical twisted fibers and closely spaced uniseriate rays of an aggregate ray. The number of rays per millimeter is 18–25 and averages 22.5. Ray parenchyma cells are lignified and some may contain crystals.

Xylem parenchyma is metatracheal, terminal, and sparingly diffuse. It is scanty in amount.

No pith flecks were observed.

### Carpinus

Eleven species of *Carpinus*, in the two sections of the genus, were studied. The two sections are very similar anatomically and will not be considered separately.

Growth rings occur in all species. They are typically wavy in outline because of the presence of aggregate rays (fig. 34). They range in width from 125 $\mu$ to 4 mm. and are mostly wide, except in *C. turczaninowii*, where they are closely spaced.

Only fiber-tracheids occur in *Carpinus*. The thickness of the radial wall ranges from very thin to thick, mostly thin to thick. Very thick-walled fibers occur at the boundary of growth rings. These latter fibers are not found in sharply delimited tangential rows, as they are in the Betuleae; instead, there is a gradual transition from the thin to thick-walled fibers of the early wood to the very thick-walled, last-formed fibers of the late wood (fig. 27). In *C. turczaninowii* some of the last-formed fibers of the growth ring are gelatinous. No gum deposits occur in the fibers.

*Carpinus* is diffuse-porous. The vessels are solitary, in pore clusters, or pore multiples of 2–10, mostly 2–6 vessels.

The vessels are angular and thin walled in all species.

The number of vessels per square millimeter is 15–194, most frequently 30–100, and the average of the means of all species is 54 (very numerous). *C. turczaninowii* has a mean number of vessels per millimeter of 124, considerably over the average of means for the genus, and a figure that might be interpreted as one from a young-wood specimen. The specimen examined had over twenty-five closely spaced growth rings, however, and so should be considered mature wood. The same is true of *C. erosa* (fig. 34), which has a mean vessel number of 115, but which is apparently also a mature-wood specimen. If these two species are excluded, the average of means of vessel number becomes 41/sq mm. (very numerous).

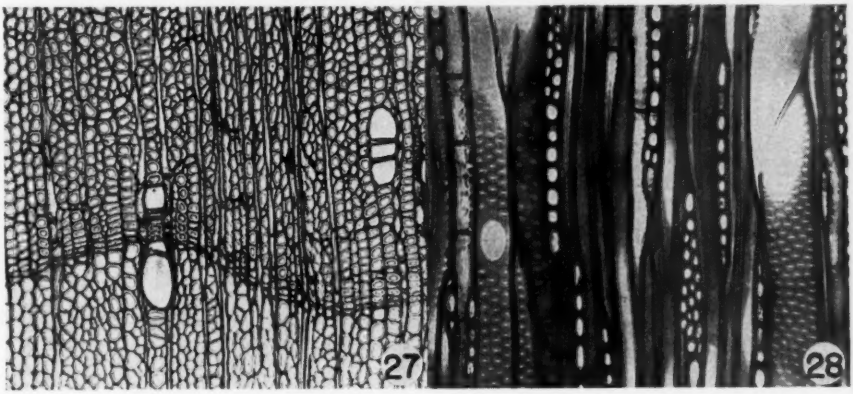
Vessel diameter is 8–106 μ, most frequently 25–80 μ, with an average of means of 49 μ (very small).

Means of length of vessel elements range from 505 to 949 μ, with an average of means of 656 μ (medium sized).

The intervascular pitting is alternate (figs. 28, 29); the pits are circular or pentagonal owing to crowding and average approximately 8 μ in diameter (medium sized). Spiral thickenings are found in the vessels (fig. 30).

Perforation plates show considerable variation among the species. In some (*C. cordata*, *C. erosa*) they are exclusively scalariform with few to intermediate widely spaced bars. In some (*C. turczaninowii*) both scalariform perforation plates with few to intermediate widely spaced bars and simple perforations with vestigial bars occur (fig. 30). In others (*C. betula*, fig. 28; *C. carpinoides*) simple perforations occur, either without vestiges of bars or with some vestigial bars, but with no evidence of complete bars. In those species with scalariform perforation plates the plates are mostly bordered. The angle of the end wall is 5°–45°, mostly 15°–35°.

Gum deposits are absent from the vessels. Tyloses occur in 5 species. They are few in a cross section and few to many per vessel. In most species they are thin




FIGS. 27–28.—Fig. 27, *Carpinus betulus:* cross section to show aggregate ray and abundant metatracheal parenchyma. ×90. Fig. 28, *C. betulus:* tangential section showing simple perforation and medium-sized, alternate intervascular pits. ×135.

FIGS. 29–35.—Fig. 29, *Carpinus carpinoides:* tangential section with alternate, angular, intervascular pits. ×550. Fig. 30, *C. turczaninowii:* radial section showing bordered scalariform and vestigial scalariform perforation plates. ×525. Fig. 31, *Ostrya carpinifolia:* radial section showing vestigial scalariform perforation plate. ×685. Fig. 32, *Carpinus tschonoskii:* radial section to illustrate crystals in ray parenchyma. ×90. Fig. 33, *C. cordata:* tangential section showing thin-walled tyloses in one vessel. ×85. Fig. 34, *C. erosa:* cross section showing poorly developed aggregate rays and very numerous vessels. ×15. Fig. 35, *Ostrya liana:* cross section. ×80.

walled (fig. 33), but in *C. turczaninowii* they are heavily lignified and thick walled, giving a cellular appearance to the vessels (figs. 36, 37).

Vascular rays are homogeneous I (figs. 28, 33). They are 1–5 cells wide, mostly 1–3 cells. The uniseriates are 1–44 cells high, mostly frequently 6–20. The multiseriates are 4–77 cells high, mostly 10–40 cells. Number of rays per millimeter is 9–24 and averages 15. Crystals occur in the ray cells of all species (fig. 32). Aggregate rays occur in all species. However, they are poorly developed in some species (fig. 34), as in *Ostryopsis*.

Xylem parenchyma is metatracheal, terminal, and sparingly diffuse in all species. It is abundant in most species, though in *C. erosa* it appears to be scanty because of the great number of vessels. The metatracheal bands are also somewhat indistinct in this species. In other species the metatracheal bands are 2–8 cells long in irregular tangential rows. Sieve-pitting occurs between adjacent parenchyma cells.

Pith flecks occur in *C. tschonoskii* and *C. yedoensis*.

### Ostrya

Growth rings occur in all 4 species of *Ostrya* studied; they are essentially similar to those of *Betula*—i.e., not wavy in outline and with several rows of very thick-walled fibers at the boundary of the growth rings. They range in width up to 2 mm. but are mostly narrow.

Only fiber-tracheids are found. They occur typically in small patches. These patches contain vascular rays, are devoid of vessels, and thus simulate short aggregate rays but are not continuous from one growth ring to another. The fiber walls range in thickness from thin to thick, and very thick at the boundary of the growth rings (fig. 35). Gelatinous fibers and gum deposits in the fibers occur in *O. liana*.

Pore distribution is solitary and in multiples of 2–7, mostly 2–4. The vessels are angular to circular in 2 species and angular in 2 species; all are thin walled.

Number of vessels per square millimeter is 28–138, with an average of means of 73 (very numerous). Diameters of vessels are 13–95 $\mu$, with an average of means of 51 $\mu$ (moderately small). Mean lengths are 541–813 $\mu$, with an average of means of 623 $\mu$ (medium sized).

Intervascular pitting is alternate and crowded. The pits are large and circular. Spiral thickenings occur in the vessels of all species (fig. 42).

Perforation plates are simple with vestiges of scalariform in all species (fig. 31). Vestigial scalariform perforation plates are common, and scalariform perforation plates may occur occasionally in the smaller vessels.

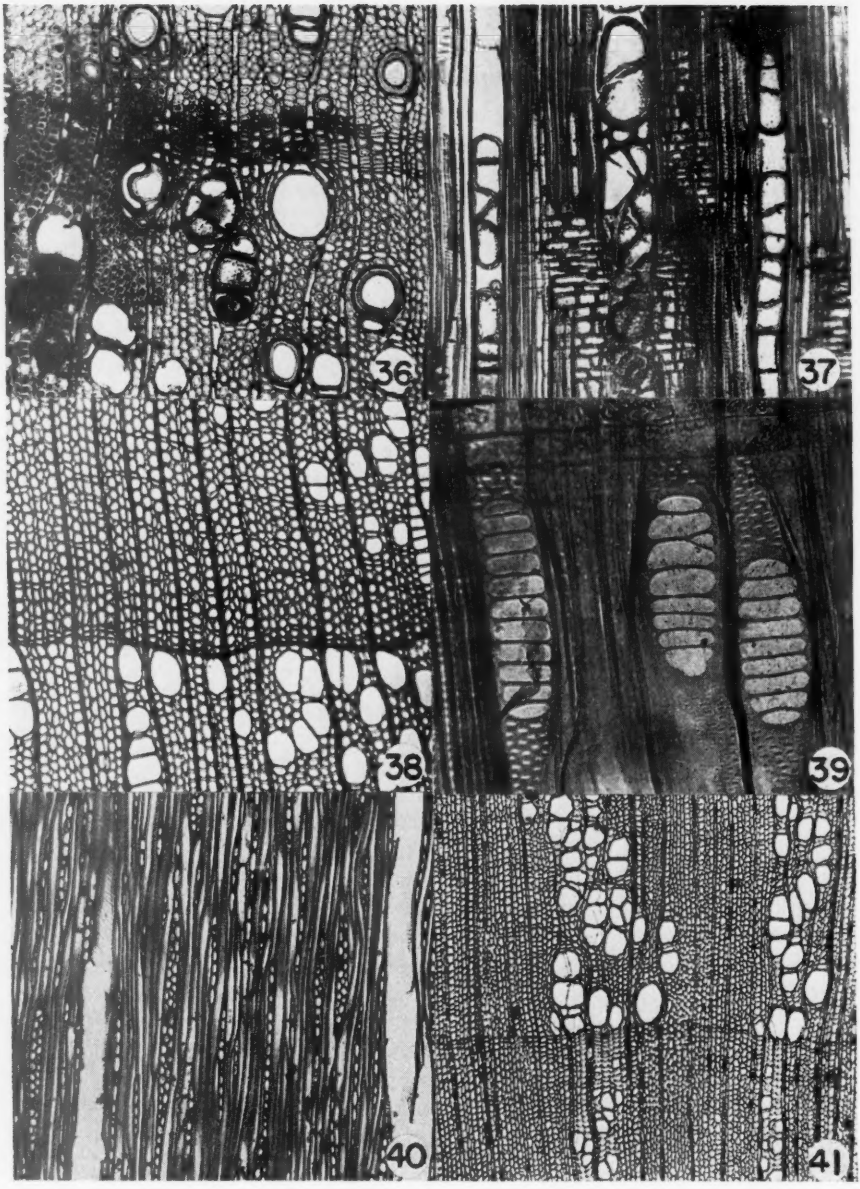
The angle of the end wall is 10°–50°, mostly 20°–40°.

Gum deposits are lacking in the vessels. Thin-walled tyloses occur in *O. liana* and *O. virginiana*. They range from few to many per vessel and are few in a cross section.

Vascular rays are homogeneous I but tend toward heterogeneous IIB in *O. japonica*. They are 1–5 cells wide, mostly 1–3 cells. Uniseriate rays are 1–38 cells high, mostly 10–25 cells. Multiseriate rays are 6–86 cells high. The number of rays is 14–32 per millimeter and averages 16.5. Aggregate rays are lacking. Crystals occur in the ray cells of all species except *O. liana*.

Xylem parenchyma is metatracheal, terminal, and sparingly diffuse in all species. In *O. liana* the parenchyma is scanty but is abundant in the other species.

Pith flecks occur in *O. japonica*.




FIGS. 36–41.—Figs. 36, 37, *Carpinus turczaninowii:* lignified tyloses in vessels. Fig. 36, cross section. ×85; Fig 37, radial section. ×90. Fig. 38, *Corylus americana:* cross section showing vesselless fibrous tract, and numerous, angular vessels in pore multiples, pore clusters, and solitary pores. ×75. Fig. 39, *C. avellana:* radial section showing scalariform perforation plates with intermediate number of bars. ×265. Fig. 40, *C. rostrata:* tangential section illustrating heterogeneous IIB rays. ×100. Fig. 41, *Ostryopsis davidiana:* cross section. ×55.

### Corylus

Six species of *Corylus* were studied.

Growth rings occur in all species; they are similar to those in *Carpinus*—wavy in outline owing to the presence of aggregate rays. They range in width up to 3.5 mm. and are mostly fairly wide.

The fibrous elements are exclusively fiber-tracheids. The thickness of the radial wall ranges from very thin to thin, with one or two rows of very thick-walled fiber-tracheids at the boundary of the growth rings. No gelatinous fibers were found.

Pore distribution is solitary, pore clusters, and pore multiples of two to many vessels; the vessels occur in flamelike groups as in *Ostryopsis*. The vessels are numerous in the early wood and become less numerous toward the boundary of the growth ring. They are angular and thin walled in all species (fig. 38).

The number of vessels per square millimeter is 75–500, with an average of means of 170 (very numerous). The diameter of the vessels is 11–63 $\mu$, with an average of means of 32 $\mu$ (very small). Average length is 366–596 $\mu$, with an average of means of 520 $\mu$ (medium sized).

Intervascular pitting is alternate. The pits are large and circular or are angular when crowded. Spiral thickenings occur in the vessels of all species.

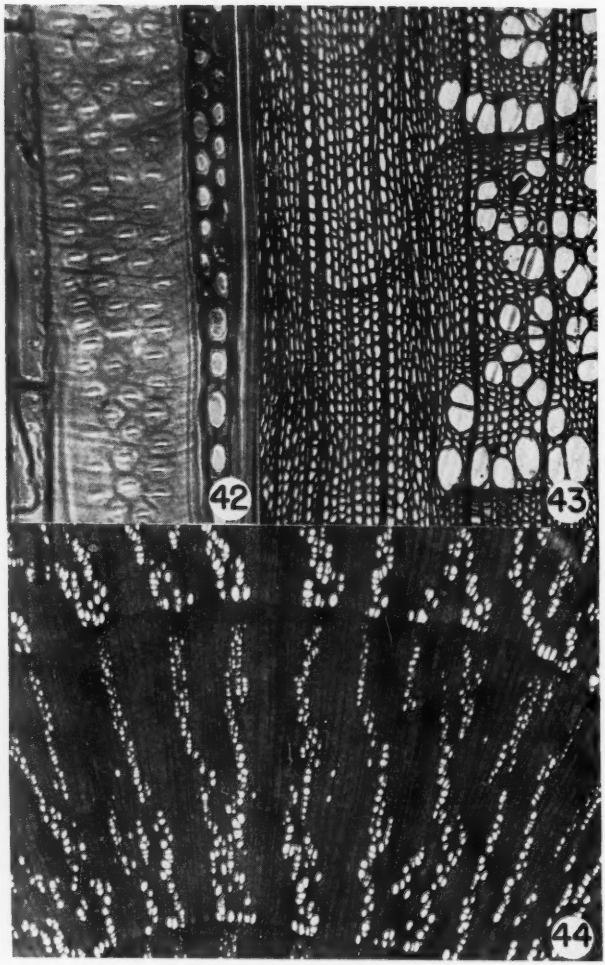
Perforation plates are scalariform with an intermediate number of bars. The perforations are wide in all species (fig. 39). They are bordered in *C. colurna* but not bordered in the other species.

The angle of the end walls of the vessel elements is 10°–35°, mostly 10°–25°.

The vascular rays are heterogeneous IIB, but there is considerable tendency to homogeneous I (fig. 40). The rays are 1–4 cells, mostly 1–3 cells, wide. The uniseriate rays are 1–42 cells high, mostly 10–20 cells; the multiseriate rays are 6–32 cells high. Aggregate rays are present in all species (fig. 43) though poorly developed in some. The number of rays per millimeter is 13–20 and averages 15.5. Crystals are lacking in the ray cells of *C. avellana* and *C. colurna* but are present in the ray cells of the other species.

Xylem parenchyma is terminal, metatracheal, and occasionally diffuse in all species; it is relatively abundant (fig. 38).

The four genera just described—*Ostryopsis*, *Carpinus*, *Ostrya*, and *Corylus*—constitute the tribe Coryleae. The anatomical characteristics of this tribe may be described as follows: The fibrous elements consist entirely of fiber-tracheids, whose walls vary in thickness from very thin to very thick at the boundary of the growth rings and are mostly thin to thick walled. Pore distribution is a combination of solitary, pore clusters, and pore multiples, except in *Ostrya*, where no pore clusters occur. The vessels are angular in most species, with a tendency toward circular in *Ostrya;* all are thin walled. Number of vessels per square millimeter is very numerous in all genera; in *Carpinus* it tends toward moderately numerous. Vessel diameter is moderately small in *Ostrya*, very small in the other three genera. Mean length of vessel elements is medium sized, to moderately short in *Ostryopsis*. Intervascular pitting is exclusively alternate; the pits are medium sized, round, or angular owing to crowding. Spiral thickenings may occur in many of the vessels. The types of perforation plates vary among the genera: in *Ostryopsis* and *Corylus* they are exclusively scalariform, in *Ostrya* simple with vestiges of scalariform, and in *Carpinus* they range from scalariform to simple with vestiges of scalariform. Tyloses occur in the vessels of *Carpinus* and *Ostrya*. Vascular rays are heterogeneous IIB in




FIGS. 42–44.—Fig. 42, *Ostrya carpinifolia:* radial section showing alternate intervascular pitting and spiral thickenings in vessel element. ×410. Fig. 43, *Corylus americana:* cross section with portion of aggregate ray. ×100. Fig. 44, *Ostryopsis davidiana:* cross section showing vessels in flamelike groups and aggregate rays. ×30.

*Ostryopsis* and *Corylus* but homogeneous I in *Carpinus* and *Ostrya*. Aggregate rays occur in all genera except *Ostrya*. Crystals occur in the ray cells of all genera. Xylem parenchyma is terminal, metatracheal, and sparsely diffuse in all genera and is relatively abundant in amount. Both ray parenchyma and xylem parenchyma cells are lignified. Pith flecks occur in *Carpinus* and *Ostrya*.

A summary of anatomical characteristics in all six genera is given in table 4.

TABLE 4

SUMMARY OF SALIENT ANATOMICAL FEATURES OF WOOD OF GENERA OF BETULACEAE

| | | *Betula* | *Alnus* | *Ostryopsis* | *Carpinus* | *Ostrya* | *Corylus* |
|---|---|---|---|---|---|---|---|
| Vessels | Mean number of vessels/sq mm | 42* | 74* | 175 | 41 | 73 | 170 |
| | Mean vessel diameter (μ) | 67* | 52* | 33 | 49 | 51 | 32 |
| | Mean vessel-element length (μ) | 761* | 716* | 351 | 670 | 623 | 520 |
| Intervascular pitting | Transitional | ..... | s† | ..... | ..... | ..... | ..... |
| | Opposite | s | s | ..... | ..... | ..... | ..... |
| | Alternate | + | + | + | + | + | + |
| Perforation plates | Scalariform | + | + | + | s | ..... | + |
| | Simple and vestiges of scalariform | ..... | ..... | ..... | s | + | ..... |
| | Simple | ..... | ..... | ..... | s | s | ..... |
| Number of bars in perforation plates | Many | + | + | ..... | ..... | ..... | ..... |
| | Intermediate | s | + | + | s | ..... | + |
| | Few | s | ..... | ..... | s | ..... | ..... |
| Spiral thickenings in vessels | | ..... | ..... | + | + | + | + |
| Fibers | Tracheids | + | + | ..... | ..... | ..... | ..... |
| | Fiber-tracheids | + | + | + | + | + | + |
| Vascular rays | Homogeneous I | + | + | ..... | + | + | ..... |
| | Heterogeneous IIB | ..... | ..... | + | ..... | ..... | + |
| Aggregate rays | | s | + | + | + | ..... | + |

* Mature-wood specimens only.

† s = Present, but not predominant; + = characteristically present in all species.

A brief general description of the wood of the entire family follows: All the Betulaceae have diffuse-porous wood, with vessels in pore clusters, pore multiples, or solitary. The vessels are angular, thin walled, very numerous, medium sized, and very small to moderately small in diameter. The perforation plates are scalariform, simple with vestiges of scalariform, or occasionally simple. Spiral thickenings may occur in the vessels (Coryleae) or may be lacking (Betuleae). Tyloses are lacking in most genera. Intervascular pitting is opposite (some) and alternate (mostly), small to medium sized. Fibrous elements are tracheids or fiber-tracheids. Vascular rays are homogeneous I or heterogeneous IIB, 1–5 cells wide and 1–80 cells, mostly 5–60 cells high. Crystals are abundant in the ray cells of most genera. Parenchyma distribution is diffuse, metatracheal, and terminal and is moderately abundant in amount. Pith flecks are common.

## Discussion

### Trends of specialization indicated by wood anatomy

From the description of the genera just presented it appears that the woods of the Betulaceae possess a series of specialized anatomical characteristics which indicate that the family does not occupy a primitive position.

Certain levels of specialization among the genera become apparent in applying the dicta referred to earlier to the various anatomical features of the wood. *Betula* is anatomically a natural genus whose characters present a moderately high level of development. Indicative of specialization are the homogeneous I rays, alternate intervascular pitting, and vessels mostly in pore clusters and pore multiples. On the other hand, there is an array of unspecialized characters in such features as small, thin-walled, angular vessels, diffuse-porosity, scalariform perforation plates with, mostly, many narrow bars, lack of libriform wood fibers, and sloping end walls to the vessel elements. Many of these characters occur without change throughout the family. Intermediate is the presence of fibertracheids as well as tracheids, considerable metatracheal and terminal wood parenchyma together with diffuse parenchyma, and medium-sized vessel elements.

There is also considerable anatomical consistency among the sections and subsections of this genus. The section Betulaster is anatomically the more advanced: the perforation plates have few to intermediate wide bars; the number of vessels per square millimeter is few, and they are large in diameter; and fibertracheids are more numerous than tracheids.

In considering the wood of members of the subsection Nanae there is the problem of differentiating the unspecialized anatomical characters of the young, immature wood of these shrubby forms from those which might be truly primitive for the genus. They have several characters which are of low anatomical level for the genus: very numerous, small, angular, thin-walled vessels whose perforation plates have many narrow bars and a predominance of tracheids. There are also occasional opposite intervascular pitting, an unspecialized character, and short vessel elements, a relatively advanced character; in the latter instance, however, the young age of the specimens probably indicates that the fusiform initials have not yet reached their mature length. Aggregate rays are found in all the natural species but not in the two hybrids included in this subsection. *B. michauxii* is unique in its almost complete lack of multiseriate rays. That most of the unspecialized characters may be truly primitive and not entirely owing to an immature sample is indicated by comparison with a mature-wood specimen of *B. pumila* with over twenty-five visible growth rings; this specimen is essentially the same anatomically as the younger specimens, except that the number of vessels tends to be less in the older growth rings, and the diameter of the older vessels is slightly greater; no aggregate rays are present in this specimen.

Two hybrids, *B. jackii* and *B. sandbergii*, are placed in this subsection. Anatomically, each appears intermediate between the two respective subsections of its parents in such characters as vessel number, diameter, and length. In the abundance and types of parenchyma each resembles the subsection Nanae; and in the lack of aggregate rays, the subsection of its other parent. These findings are essentially the same as those reported

by COUSINS (25) for the wood of *B. jackii*.

Anatomically, the two subsections Costatae and Albae are close to each other and intermediate between the two subsections previously considered. The subsection Costatae is perhaps the more advanced of the two. Diameter of the vessels is somewhat greater, although the number of vessels is less than in the subsection Albae. Parenchyma is more abundantly metatracheal in subsection Costatae but mostly diffuse in Albae. In the subsection Costatae 5 species have many bars in the perforation plates, 5 have intermediate to many, and 1 has few to intermediate bars, while in the Albae 7 species have many bars, 7 have intermediate to many, and none has few to intermediate. Also, in the subsection Costatae only 1 species has exclusively narrow bars, 7 have narrow to wide bars, and 3 have wide bars; in the Albae 6 species have narrow bars, 7 have narrow to wide bars, and only 1 has wide bars. The subsection Albae, however, is more advanced in its shorter mean length of vessel elements. In all these characters, except vessel-element length and parenchyma distribution, these two subsections are intermediate between the subsection Nanae, all of whose species have perforation plates with many narrow bars, and the subsection Acuminatae, having intermediate to few wide bars in the perforation plates.

*Alnus* has certain specialized characters which are not found in *Betula:* a greater number of species has only fibertracheids, and there is a tendency toward loss of multiseriate rays. KRIBS (47) has shown that the homogeneous III condition is specialized; although *Alnus* cannot be said to have homogeneous III rays, it closely approximates this condition. Yet, there are many anatomical characters which are more primitive than those found in *Betula:* opposite intervascular pitting in some species; a greater number of species whose perforation plates have intermediate to many bars, and none with few to intermediate bars; bars mostly narrow; relatively more diffuse than metatracheal parenchyma, and terminal parenchyma not prominent; and vessels more numerous and smaller, though vessel-element length is about the same in both genera.

Since one section of *Alnus* (Cremastogyne) is poorly represented, and another (Clethropsis) not at all, no attempt will be made to indicate trends of specialization among the sections. However, certain differences between the sections Alnobetula and Gymnothyrsus can be pointed out. In the section Alnobetula aggregate rays are absent, except in the first few growth rings, where they are poorly developed. Also, in this section there is mostly opposite intervascular pitting, with oblong pits whose apertures are slitlike, and only occasional alternate pitting. In the section Gymnothyrsus, on the other hand, aggregate rays occur in most species; in some specimens they might be widely spaced and thus not included in the sections examined. Alternate vascular pitting is not uncommon, and the pits are generally circular, with round apertures; there is also some opposite pitting, especially in the younger wood. In this section *A. acuminata* is somewhat anomalous; its vessels are moderately few in number (9/sq mm) and show a tendency to become round; aggregate rays are very poorly developed (fig. 21); growth rings are absent (this is a subtropical species); and vessel elements are moderately long.

The two available specimens of *Ostryopsis*, both of which are young, show no distinguishable anatomical differences from the wood of *Corylus*, and the two

genera may be considered together. This similarity in wood structure is somewhat surprising in view of the differences in floral morphology. However, this may be accounted for in part by the relative immaturity of the wood of *Ostryopsis*, though several of the woods of *Corylus* examined were also young.

In each genus the number of bars in the perforation plates, which are all wide, is intermediate to few. In *Ostryopsis* an occasional vestigial bar is found, indicating a trend toward simple perforations by the loss of bars. Each genus has fiber-tracheids exclusively, spiral thickenings in the vessels, heterogeneous IIB rays, considerable metatracheal parenchyma with a tendency toward reduction in the amount of diffuse parenchyma, and alternate intervascular pitting composed of medium-sized, circular bordered pits. Except for heterogeneous rays all these characters are more specialized than those found in the Betuleae. Even the heterogeneous rays show a tendency toward homogeneity. Aggregate rays are not well developed in *Ostryopsis* or in the early growth rings of *Corylus;* they become more prominent in the more mature wood of both genera, however.

*Carpinus* shows further reduction in the scalariform perforation plates. Some species have scalariform perforation plates with intermediate to few bars, while in other species this type of perforation is replaced by the simple type. However, vestiges of the bars of the scalariform perforation plates are found in all species. An advance beyond *Ostryopsis* and *Corylus* is also found in the type of vascular rays, which are homogeneous I. *Carpinus* also has those other features of anatomical specialization found throughout the tribe Coryleae: spiral thickenings in the vessels, alternate intervascular pitting, fiber-tracheids, and metatracheal and terminal parenchyma in addition to some diffuse parenchyma. Aggregate rays are well developed in *Carpinus*.

Finally, *Ostrya* is the most specialized member of the tribe and of the family as a whole. Its perforation plates are almost exclusively simple. Scalariform perforation plates with few (1–3) bars occur occasionally in the smaller vessels, and vestigial bars are found in some of the simple perforations in all species; no species is found without simple perforation plates, however. As in *Carpinus*, homogeneous I rays are present; aggregate rays are lacking, however. If all the other specialized characters of the tribe which are found in *Ostrya* are considered, it is obvious that this genus has reached a relatively high anatomical level.

Summarizing the results of the above discussion, the phylogenetic sequence of the genera can be indicated. The Betulaceae are an anatomically natural and closely knit family composed of two tribes, also homogeneous, at two levels of specialization. One tribe, the Betuleae, is relatively the least specialized; the other, the Coryleae, has an array of characters which are anatomically of greater specialization. Within each tribe certain trends of specialization are also evident. Thus, in the Betuleae, *Alnus* and *Betula* are very similar in most anatomical characters. *Alnus*, however, is undoubtedly the less specialized, with perforation plates which have numerous closely spaced bars. The tendency to reduce the number of bars and to widen them is carried on in *Betula*, with the furthest extreme in this genus in the subsection Acuminatae. The more primitive opposite pitting found in some species of *Alnus* tends toward alternate in that genus and is completely so in *Betula*. In the Coryleae, certain relatively more spe-

cialized characters appear. In this tribe *Ostryopsis* and *Corylus* are at the same anatomical level. They have further reduced the number of bars in the perforation plates, have no tracheids, and spiral thickenings are found in the vessels; however, they have heterogeneous rays. *Carpinus* is more specialized in having homogeneous rays and a pronounced tendency toward simple perforation plates. *Ostrya* reaches the peak of specialization in the family, with simple perforations occurring almost exclusively in addition to the other characters indicative of specialization in this tribe. The relationships of the genera as suggested by this study are shown in figure 45.

### Evidence from other fields of investigation

The question now arises as to the phylogenetic position and possible ancestry of the Betulaceae. As has been shown, the Betulaceae are anatomically not a primitive family but have many moderately specialized anatomical characters. Yet, of the so-called "Amentiferae," they probably stand among the lowest. Moseley (**52**) has shown that the Casuarinaceae, once presumed to be primitive members of the "Amentiferae," are moderately specialized anatomically. In many respects they are more specialized than the Betulaceae, possessing more nearly circular vessels, mostly simple perforation plates, fairly short vessel elements with some end walls at 90°, larger vessels, and spiral thickenings in some of the fibers and most of the vessels. In certain respects, however, they are more primitive—as in the possession of a greater percentage of tracheids and heterogeneous rays. Thus, we cannot consider the Casuarinaceae as an ancestral type for the Betulaceae but must find some group less specialized than both of these families. That this might be a hamamelidaceous stock has been indicated by Tippo (**74**). The present-day Hamamelidaceae are anatomically very unspecialized, standing close to the Magnoliaceae in many of their primitive characters: tracheids almost exclusively; solitary, angular, thin-walled, extremely small vessels; diffuse-porous wood; exclusively scalariform perforation plates; very oblique end walls to the vessel elements; scalariform, transitional and opposite intervascular pitting; long vessel elements; heterogeneous vascular rays; and mostly diffuse parenchyma. It is obvious that the Betulaceae are anatomically somewhat more advanced than the Hamamelidaceae, and it is possible that the former were derived from an ancestral stock close to the present-day Hamamelidaceae. To substantiate

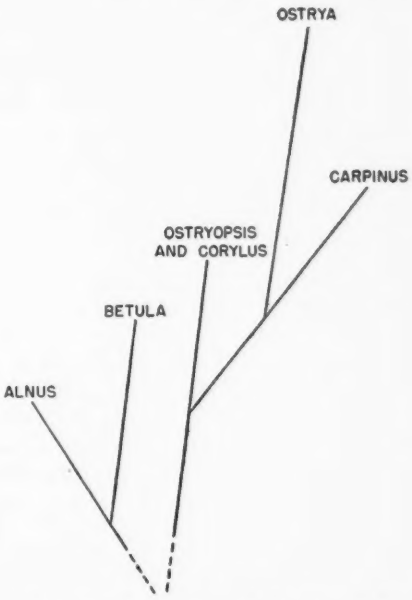



Fig. 45.—Phylogenetic relationships of Betulaceae as indicated by their wood anatomy.

such an argument, evidence from other fields of study should also be examined.

FLORAL AND INFLORESCENCE MORPHOLOGY.—ABBE (1, 2) has investigated the floral and inflorescence anatomy and morphology of the Betulaceae and has found much that substantiates the conclusion that the Betulaceae are not a primitive family. Several factors have modified the basic plan of the florets and inflorescences: reduction of parts and loss of entire florets, fusion of parts, shortening of the internodes, and compression within the ament. From his data ABBE hypothecated ancestors with florets on the trimerous plan: hermaphrodite florets with a hexamerous perigone, hexamerous androecium, and an inferior tricarpellary ovary. Likewise, the cymule would be composed of a series of racemosely arranged cymose inflorescences, each composed of a median and two lateral triflorous groups and their bracts. For an ancestral type ABBE tentatively suggested the Fagaceae; wood anatomy, however, does not support this contention, since the wood of the Fagaceae is anatomically considerably more specialized than that of the Betulaceae.

GAMETOPHYTE MORPHOLOGY.—Certain pertinent features of the gametophyte structure should be pointed out. Studies in the development of the female gametophyte and the course of the pollen tube in the Betulaceae were made following TREUB's discovery of chalazogamy in *Casuarina* in the latter part of the past century (14, 15, 53–58).

In *Corylus* a massive "sporogenous tissue," comparable with that of *Casuarina* except that it is not so sharply delimited from the remaining cells of the nucellus, gives rise to several megaspore mother cells, most of which can form embryo sacs. It is reported that some of the cells of the sporogenous tissue give rise to tracheids (55), also as in *Casuarina*, though in the light of SWAMY's (73) study of *Casuarina* these tracheids might be partly an extension of the vascular trace which supplies the placenta. Numerous megaspores arise, all of which can give rise to embryo sacs, but usually it is only the basal one which develops. The embryo sac is monosporic and of the normal type. WOODWORTH (85) has described free nuclear division of endosperm initials previous to division of the egg cell in *Alnus rugosa*, but this is not the usual situation. The egg apparatus is not completely formed at the time of pollination; some 3 months elapse from pollination until the pollen tube reaches the embryo sac. At the time of fertilization the embryo sac lengthens somewhat, though not to such an extent as in *Casuarina*.

In all the Betulaceae the pollen tubes penetrate the nucellus through the chalazal end of the ovule to reach the embryo sac. SWAMY (73), in a classification of the types of chalazogamy, pointed out two different types in the Betulaceae: an extravesicular type in the Betuleae (Betulaceae), in which the pollen tubes reach the egg by traveling outside the embryo sac that is to be fertilized, and an intravesicular type in the Coryleae (Corylaceae), in which the pollen tube reaches the egg by direct penetration of the embryo sac at its chalazal end. In most of the Betulaceae the pollen tubes branch; in some genera the sperm nucleus which is to unite with the polar nuclei travels through one of the branches.

Many of the abnormalities in the gametophytic development of the Betulaceae noted above are found in a wide range of angiosperm families and are probably of slight phylogenetic significance. Thus, chalazogamy, to which considerable phylogenetic value was attached at one time, is now also known in

the Casuarinaceae and Juglandaceae and, in modified form (mesogamy), in the Cucurbitaceae, Rosaceae, Ulmaceae, and Cannabinaceae. Accordingly, chalazogamy is better interpreted as an aberrant phenomenon and probably of little evolutionary importance. A massive sporogenous tissue is also found in the Rosaceae, Malvaceae, and Casuarinaceae; numerous megaspores occur as well in the ovules of the Ranunculaceae, Rosaceae, Onagraceae, Compositae, Fagaceae, Umbelliferae, Casuarinaceae, and Hamamelidaceae. Several embryo sacs occur in one ovule of both Betulaceae and Casuarinaceae. Branching pollen tubes also occur in the Fagaceae, Juglandaceae, Casuarinaceae, Ulmaceae, Cannabinaceae, Araceae, and Amaryllidaceae (**73**). It will be noted that many of these unusual features of ovule and gametophyte morphology are found both in the Betulaceae and in the Casuarinaceae. This may indicate phylogenetic relationship between the two families, possibly common ancestry. It is perhaps significant, in view of the suggestion of the Hamamelidaceae as possible progenitors of the Betulaceae, that in *Hamamelis* there are also numerous megaspores in the ovules.

CYTOLOGY.—The Betulaceae have been examined cytologically by WOODWORTH (**83–87**). The haploid chromosome number of *Betula*, *Alnus*, and *Corylus* is 14, of *Carpinus*, *Ostrya*, and *Ostryopsis*, 8. WOODWORTH stated that the basic number for the family is probably 7, though no species has been found with this number. Those genera with 8 chromosomes could have arisen by the duplication of 1 chromosome, and those with 14 by duplication of the entire original genome of 7. Other chromosome numbers that have been reported (**32, 33, 77**) are 11, 21, 28, 32, 35, and 42. Considerable polyploidy occurs among the species, and hybrids are common, especially in *Betula*. On the basis of chromosome number, *Corylus* appears to be aligned with *Betula* and *Alnus*, though the wood anatomy does not substantiate such a grouping.

PALEOBOTANY.—The fossil record, usually so imperfect when concerned with the phylogeny of the angiosperms, is not entirely inconclusive in the case of the Betulaceae. Some 104 named fossil species and 19 varieties of leaf impressions and woods have been recorded by KNOWLTON (**46**) and LAMOTTE (**49**) from the Mesozoic and Cenozoic of North America. EDWARDS, in the *Fossilium Catalogus* (**27**), listed 20 named and 6 unnamed species of fossil betulaceous woods. The stratigraphic distribution of the members of the family is of interest, since, as PRANTL (**60**) and ARNOLD (**3**) have pointed out, *Alnus* and *Betula* are found from the Cretaceous onward, while members of the Coryleae do not appear in the geologic record until the Tertiary. This obviously supports the data derived from anatomical evidence as to the relatively less specialized position of the Betuleae in the family. However, little is to be gained from the fossil record as to the relative position of the family as a whole, since it appears suddenly on the geological scene along with other angiosperms which are taxonomically both primitive and advanced.

POLLEN MORPHOLOGY.—Pollen morphology offers little in the way of phylogenetic evidence in the Betulaceae. The pollen grains of the Betulaceae are acolpate, but WODEHOUSE (**82**) stated that this condition is one which is contingent on the anemophilous habit and is found in many entirely unrelated genera of anemophilous plants. The acolpate condition is probably derived by the thin-

ning-down of a thick exine to such a point that it can no longer support furrows and in this respect might be considered specialized.

NODAL ANATOMY.—Three types of nodal anatomy, based on the number of leaf traces and gaps, have been described by SINNOTT (69). The trilacunar condition has been considered the more primitive type, from which the unilacunar and the multilacunar are presumably derived. All the Betulaceae have trilacunar nodes. They are unspecialized in this respect and similar to the Hamamelidaceae, whose nodes are also trilacunar.

EXTERNAL MORPHOLOGY.—Here may be mentioned the occurrence of transverse lenticels on the stems of the Betulaceae; WETMORE (79, 80) stated that this type is more unspecialized than vertical lenticels. Also to be considered is the anemophilous condition of the flowers of the Betulaceae, for ROBERTSON (67) has shown that anemophily is derived from entomophily and should be considered specialized.

SPORNE (72) has presented additional evidence for the relatively moderate specialization of the Betulaceae in an "advancement index." He listed twelve primitive morphological and floral characters for all families of dicotyledons, all of which are correlated. From these he determined the ratio of advanced to primitive characters within a family, to arrive at the advancement index. Thus, a family with a low advancement index would have many primitive characters among the twelve listed. The Betulaceae have an advancement index of 40, based on these characters, which is considerably above the lowest advancement index of 8. Other pertinent advancement indices are: Magnoliaceae, 12; Fagaceae, 30; Casuarinaceae, 50; Hamamelidaceae, 54. Anatomical features of the wood and nodal anatomy are not included, however, among the characters used to determine the advancement index. Objection might also be raised to considering unisexual flowers primitive, as SPORNE did, especially in the Betulaceae, where there is evidence of reduction from hermaphroditic flowers.

## Conclusions

The available anatomical and morphological evidence suggests that the Betulaceae are not a primitive family. Wood anatomy, especially, indicates that the Betulaceae are moderately advanced. The presence of mostly homogeneous I rays, alternate intervascular pitting, spiral thickenings in the vessels of some genera, considerable metatracheal parenchyma, and the tendency toward simple perforation plates all are indicative of specialization. However, such unspecialized characters as very numerous, small, angular, thin-walled vessels, scalariform perforations, and heterogeneous rays in some species indicate that the level of specialization is only moderately high. Based largely on a study of their aggregate rays, HOAR (39) came to the conclusion that the Betulaceae are among the most primitive dicotyledons. HOAR considered that aggregate rays were indicative of primitiveness and that from them the simpler uniseriate and multiseriate rays arose by "diffusion." Recent work, however (11, 52), has indicated that in some genera aggregate rays may arise phylogenetically by the aggregation of multiseriate and uniseriate rays or that, in other genera, the aggregate rays may give rise to the multiseriate and uniseriate rays by dissection; in other words, the evolutionary series may be read in either direction. Evidence from other fields of investigation substantiates the specialized position of the family and the

suggestion that the Betulaceae might have originated from some hamamelidaceous stock.

Of the various phyletic schemes involving the dicotyledons, the most tenable in the light of present evidence are those which have been based on the ranalian hypothesis, and, of these, HUTCHINSON'S (41) scheme comes closest to agreeing with the evolutionary position of the Betulaceae as determined by the present study. He derived the Betulaceae from the Hamamelidaceae, which in turn have come from the Rosales. This latter relationship is somewhat open to question, as the Hamamelidaceae are anatomically less specialized than the Rosales. HALLIER (36) also placed the Betulaceae near the Hamamelidaceae. BESSEY (16), however, placed the Betulaceae among the Sapindales, next to the Juglandaceae. Anatomical evidence does not support this position. HEIMSCH and WETMORE (38) have shown that the wood of the Juglandaceae is moderately advanced. It is more advanced than that of the Betulaceae and probably not on the same lines of specialization. It is of interest that BESSEY once placed the Casuarinaceae among the Sapindales but later moved them to a position closer to the Hamamelidaceae; the same could well be done with the Betulaceae. A rather fantastic proposal is that of HAGERUP (35), who considered the ovules of *Corylus* monosporangiate macrosporangia and in this respect saw a phylogenetic relationship between *Corylus* and the Lycopodiales.

Taxonomically, the Betulaceae are readily divisible into two tribes; these two tribes have sometimes been raised to family rank. Although there are some marked anatomical differences between the two tribes, the question of whether they should be made families is one which requires further research. Study of wood anatomy can point out differences and similarities between the two groups which then can be used in determining their taxonomic status.

## Summary

1. The wood anatomy of the Betulaceae has been studied comparatively in an attempt to use it in establishing the phylogenetic position of the family.

2. Descriptions are given of the wood of the six genera—*Alnus*, *Betula*, *Corylus*, *Ostryopsis*, *Carpinus*, and *Ostrya*—as well as of the two tribes in which these genera occur (Betuleae and Coryleae) and of the family as a whole.

3. The trends of specialization within the family, as evidenced by wood anatomy, are discussed. The tribe Betuleae is more primitive than the Coryleae. In the former tribe *Alnus* is less specialized than *Betula* in the possession of some opposite intervascular pitting and many-barred perforation plates. *Betula* has mostly alternate pitting and has a reduced number and wider bars in the perforation plates. In the tribe Coryleae, *Corylus* and *Ostryopsis* have spiral thickenings in the vessels; the rays are heterogeneous; the perforation plates are scalariform with few, wide bars; and the intervascular pitting is alternate. *Carpinus* shows a tendency toward simple perforations and homogeneous rays, and in *Ostrya* the perforations are almost entirely simple, with only a few scalariform perforation plates in the smaller vessels. Most of these characters of the Coryleae indicate greater specialization than in the Betuleae.

4. Evidence from other fields of investigation is introduced to indicate that the Betulaceae are moderately specialized and to suggest that the family is probably derived from some hamamelidaceous ancestor.

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# FLORAL DEVELOPMENT, SPOROGENESIS, AND EMBRYOLOGY IN THE AVOCADO, PERSEA AMERICANA

C. A. SCHROEDER

## Introduction

Within recent decades the avocado, *Persea americana* Miller,[1] has become an important crop plant in California, Florida, and other subtropical regions. Consequently, much interest has developed concerning the plant and its fruit. Some preliminary studies have been made on several phases of the anatomy of the whole plant (**4**) and of the fruit (**3**), but relatively little information on floral development exists. The present study deals with floral development, sporogenesis, and certain phases of early embryo development in the avocado.

In an early study of microsporogenesis in *P. gratissima* Gaertn. the haploid chromosome number was determined to be 12 (**1**). Cytological irregularities were also observed such as gigantic microspores which contained two or more nuclei and gave rise to polyploid pollen grains. The occurrence of ovules borne externally on the ovaries has been noted in Rome (**2**) and in California (**9**). Anatomical evidence (**7**) indicates that the six-parted perianth of the flower arises in two whorls and consists of a three-parted calyx and a three-parted corolla. In Florida (**8**) differentiation of the inflorescence continues over a period of months, but the earliest primordia of individual flowers are first identifiable in January only a few weeks before full bloom.

[1] The nomenclature is that suggested by POPENOE (**6**).

## Material and methods

Materials for the present investigation were obtained from the horticulture orchard on the Los Angeles campus of the University of California and from commercial plantings in various parts of southern California. They consisted mainly of buds, flowers, and young fruits of the Fuerte variety, although similar collections were made of varieties such as Nabal, Matney, Mexicola, Dickinson, Blake, and others. They were fixed in Navashin's solution, after which they were dehydrated and imbedded in paraffin. Sections cut 10–20 $\mu$ thick were stained with safranin and fast green, Heidenhain's iron-alum hematoxylin, or Harris' hematoxylin.

## Observations

FLORAL DEVELOPMENT.—In longitudinal section the growing apex of the individual flower primordium is first evident as a conical mass of cells in the axial of a bud scale (fig. 1*A*). This mass of meristematic cells becomes slightly flattened, and on the periphery of the disk the perianth parts appear (fig. 1*B–D*). These rudimentary perianth segments elongate and curve inward, arching over the apex of the axis. Stamen primordia arise at the same level but inside the perianth

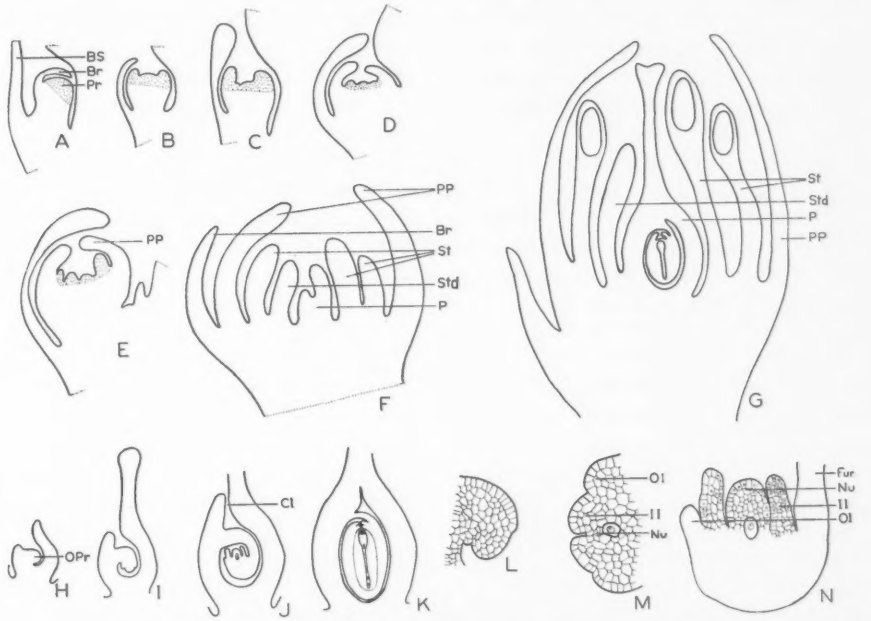


FIG. 1.—*Persea americana*. *A–G*, differentiation of flower parts. *A*, flower primordium in axil of bud scale. *B*, initiation of perianth primordia. *C–E*, initiation of stamens and staminodes. *F*, section through young flower shortly after initiation of pistil. *G*, young flower with all parts differentiated. *H–N*, differentiation of ovule and integuments. *H*, initiation of ovule in young pistil. *I–K*, later developmental stages of ovary and ovule. *L*, differentiation of inner integument in young ovule. *M*, differentiation of outer integument in young ovule. *N*, older stage of ovule with both integuments. *BS*, bud scale; *Br*, bract; *P*, pistil; *PP*, perianth part; *Pr*, bud primordium; *St*, stamen; *Std*, staminode; *OPr*, ovule primordium; *Cl*, cleft in pistil; *OI*, outer integument; *II*, inner integument; *Fun*, funiculus; *Nu*, nucellus.

parts and opposite to them (fig. 1*D*). Inside the stamen whorl appears a third set of primordia, the inner stamens or staminodia (fig. 1*E*). The pistil is the last floral organ to develop (fig. 1*F*, *G*). It first appears as a small, conical mass which by differential growth produces a cuplike structure, one side of which becomes higher than the other. The higher side gives rise to the style and stigma, and the lower, which grows more slowly, differentiates the ovule primordium on its inner surface (fig. 1*F*, *H–J*).

More rapid cell division on the upper side of the funiculus causes the ovule to bend toward the receptacle. Differentiation of integuments and nucellus occurs at this time. The inner integument appears first and is followed by the outer (fig. 1*L–N*). By continued bending the ovule eventually becomes anatropous, and the micropyle is located immediately beneath the funicular attachment (fig. 1*K*).

MEGASPOROGENESIS.—A single hypodermal archesporial cell gives rise to the megaspore mother cell and the primary parietal cell (fig. 2*A*) at about the time of initiation of the inner integument. The megaspore mother cell, distinguished by its central position, large nucleus, and deeply staining cytoplasm, enlarges to four to five times the size of the archesporial cell and becomes imbedded in several layers of nucellar tissue (fig. 2*B*).

By two successive divisions the megaspore mother cell gives rise first to two (fig. 2*C*) and then to a linear row of four megaspores of about the same size. The three micropylar megaspores soon degenerate, leaving the chalazal megaspore to function as the one-cell embryo sac (fig. 2*D*). Before the first division of its nucleus, the functional megaspore increases in size, accompanied by an increase in cell division in the surrounding nucellar tissue. Following the first gametophytic division the two daughter nuclei separate, one migrating to the chalazal end and one to the micropylar end of the elongating and enlarging embryo sac (fig. 2*E*). By two subsequent divisions, these two nuclei produce eight nuclei, a group of four at each end of the now elongate embryo sac (fig. 2*F*, *G*). One nucleus from each of the two groups migrates toward the center (fig. 2*H*). Fusion of these two polar nuclei then occurs (fig. 2*I–K*). As the embryo sac continues to elongate, the three remaining chalazal nuclei migrate further toward the chalazal end and become the antipodals. They are scattered irregularly and generally undergo degeneration very early, sometimes even before the fusion of the polar nuclei. Only traces of the antipodals remain by the time the egg apparatus is mature. The three nuclei remaining in the micropylar end of the embyo sac become the egg apparatus. The nucleus which is centrally located in respect to the micropyle becomes surrounded by a thin membrane to form the megagamete. The other two nuclei, the synergids, are lateral to the megagamete and also become enveloped by distinct membranes.

At maturity the embryo sac is imbedded in nucellar tissue. It is an elongate structure somewhat enlarged at the micropylar end (fig. 2*L*) and measures 650–950 $\mu$ in length and 25–75 $\mu$ in width.

Cytological irregularities in the embryo sac were observed rather frequently. The most common abnormalities noted consisted of cross walls, irregularity in shape, doubleness, and an increased number of nuclei.

Cross walls were found in several embryo sacs. These walls were about the thickness of the walls of the surrounding

nucellus and extended completely across the sac. Most of the cross walls were confined to the chalazal region, although in some cases they were found at about the level of the polar nuclei. Separation of the antipodal nuclei by wall formation was seen in several instances.

In abnormal embryo sacs the number of nuclei ranged from nine to twenty-five or more. In one case three nuclei were found just lateral to the egg apparatus in the micropylar region, and in another two extra nuclei were noted in the region of the antipodals in the chalazal end. The origin of these nuclei could not be determined. Other embryo sacs contained

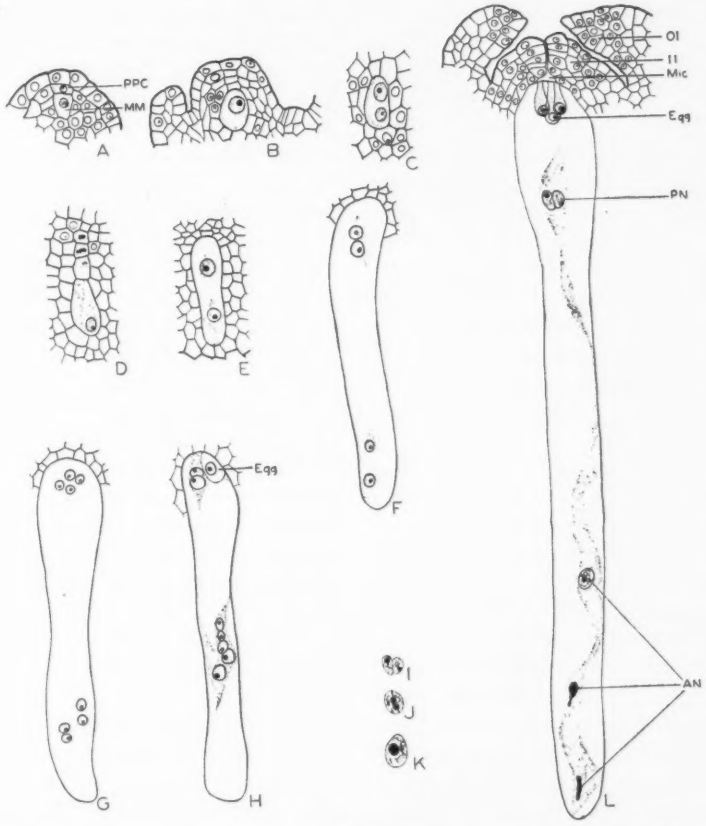


FIG. 2.—*Persea americana*. Megasporogenesis and development of megagametophyte. *A*, section through tip of developing ovule shortly after division of hypodermal archesporial cell. *B*, megaspore mother cell. *C*, daughter nuclei from first division of megaspore mother cell. *D*, linear tetrad of megaspores, showing the chalazal spore becoming functional, the micropylar three disintegrating. *E*, two-nucleate embryo sac. *F*, four-nucleate embryo sac. *G*, eight-nucleate embryo sac. *H*, eight-nucleate embryo sac at later stage showing migration of polar nuclei to center of sac and differentiation of egg cell, synergids, and antipodals. Polar nuclei are central in position in this section. *I*, polar nuclei prior to fusion. *J*, secondary nucleus, with two nucleoli. *K*, secondary nucleus with single, large nucleolus. *L*, mature embryo sac. *PPC*, primary parietal cell; *MM*, megaspore mother cell; *PN*, polar nuclei; *AN*, antipodal nuclei; *OI*, outer integument; *II*, inner integument; *Mic*, micropyle.

many small nuclei in the chalazal end. These numbered from ten to twenty-five or more in each sac. They were very small, approximately 1–3 $\mu$ in diameter, and were scattered irregularly.

An ovule containing two sacs was found in a single specimen. This corroborates the observation of polyembryony in avocado seeds.

MICROSPOROGENESIS.—In flower buds about 1 mm. in length, the first evidence of microsporogenesis appears in longitudinal section as a single hypodermal cell which contains a relatively large nucleus and stains deeply (fig. 3*A*). In rapid succession several of the adjacent hypodermal cells exhibit a similar reaction (fig. 3*B*, *C*). Continued cell division in this tissue results in a group of cells, the sporogenous tissue, four to five cells deep and six to ten cells in length just beneath the epidermis (fig. 3*D*, *E*). The cytoplasm of all these sporogenous cells stains deeply, and the single nuclei are conspicuously large.

The tapetum arises from the peripheral layer of the sporogenous tissue (fig. 3*F*). The four-nucleate condition of the cells of this tissue arises as the result of two successive nuclear divisions without ensuing cell-wall formation (fig. 3*G–I*). In the two-nucleate condition the nuclei are often elongated and flattened along the surface of contact and often show two or three nucleoli. After the second division, the four nuclei present in the cell are smaller, more nearly spherical in shape, and possess only one nucleolus each. In prepared materials sometimes the four nuclei are tightly pressed together and thus are distorted from their spherical shape (fig. 3*I*). As this nutritive layer is absorbed during the development of the microspores, the cytoplasm is reduced in amount, the nuclei undergo degeneration, and the cell walls collapse and shrink (fig. 3*J*, *K*). There are no remains of the tapetal layer in the mature pollen chamber.

Shortly after the formation of the tapetum, the spore mother cells undergo meiotic division resulting in the subsequent formation of tetrads (fig. 3*F*, *L–N*). The chromosomes have not been well observed during meiotic divisions. They are small, about 1.5 $\mu$ in length, and tend to clump together when ordinary techniques are used. In a few of the better preparations the haploid complement was determined to be twelve.

The microspores remain united in tetrads for only a short time. When they become separated, they assume a spherical shape and have a thin limiting membrane and a large nucleus with one large, spherical nucleolus. The spore wall thickens, and the exine appears (fig. 3*O*, *P*). The pollen grains may be shed in this stage. In most cases, however, the nucleus divides before the pollen is shed. The division results in a large nucleus, retaining a central position, and a smaller nucleus which lies near the periphery of the grain (fig. 3*Q*, *R*). Each nucleus, surrounded by cytoplasm, becomes incased in a thin membrane. Thus in preparations which are slightly plasmolyzed two distinct cells can be identified in the male gametophyte at this stage (fig. 3*R*).

The heavy intine of uniform thickness lies next to the cytoplasm and is covered by a thin exine with short, uniformly distributed conical spines (fig. 3*S*). The exine and intine are easily ruptured when subjected to pressure (fig. 3*T*).

The mature Fuerte pollen grain measures 27–30 $\mu$ in diameter. The intine and exine are 2 $\mu$ and less than 1 $\mu$ thick, respectively. When placed in water or IKI solution, marked swelling of the grain occurs. As there are no germinal furrows

or conspicuous germ pores in the microspore wall, germination of the pollen tube may occur by the splitting of the wall at any point on its surface. The tube nucleus precedes the generative nucleus as they migrate into the elongating pollen tube (fig. 3*U–W*). Shortly after the beginning of germination, the membrane separating the generative and tube nuclei seems to disappear so that the nuclei at least in the early part of their migration down the germ tube are enveloped by a common cytoplasm (fig. 3*U*, *V*). The ultimate fate of either generative or tube

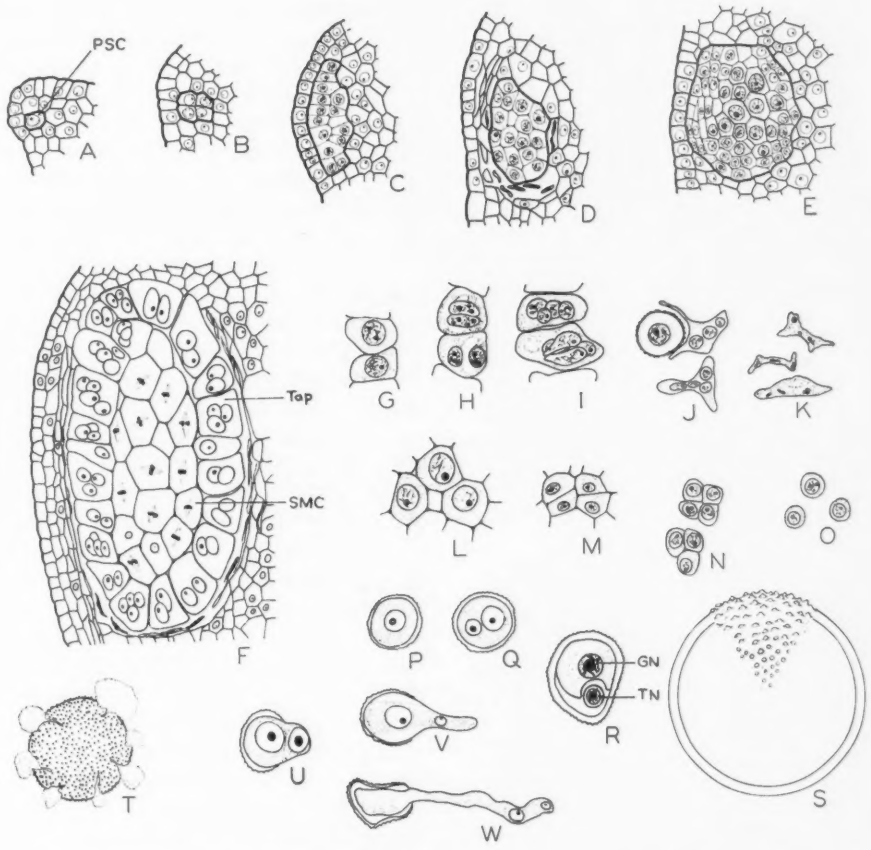


FIG. 3.—*Persea americana*. Microsporogenesis and young microgametophyte. *A*, primary sporogenous cell in young anther. *B–E*, later stages in development of sporogenous tissue. *F*, pollen sac shortly after differentiation into spore mother cells and tapetal layer. *G–I*, one-, two-, and four-nucleate stages of tapetal cells. *J*, *K*, tapetal cells being absorbed by developing microspores. *L*, microspore mother cells. *M*, microspores before second division. *N*, tetrads of microspores. *O*, *P*, young microspores. *Q*, pollen grain with generative and tube nuclei. *R*, mature pollen grain slightly plasmolyzed. *S*, portion of pollen-grain surface showing blunt, conical spines. *T*, bursting of pollen grain when subjected to pressure. *U*, pollen grain starting to germinate; only faint indication of wall separating generative and tube cells remains. *V*, *W*, germinating pollen grains. *Tap*, tapetum; *PSC*, primary archesporial cell; *SMC*, microspore mother cell; *GN*, generative nucleus; *TN*, tube nucleus.

nucleus could not be determined from the materials studied.

ENDOSPERM DEVELOPMENT.—The endosperm tissue apparently results from a triple fusion of chromosome complements, although as yet no fusion stages have been observed. In aceto-carmine smears the chromosome number in thirteen preparations ranged from 27 to 39. The chromosomes are minute (1.2 × 0.6 $\mu$) (fig. 4*A*) and tend to clump, so that they are difficult to count. The diploid complement has been reported to be twenty-four (1, 10). Thus, the observed chromosome number in endosperm tissue is close to the calculated triploid. The occurrence of three nucleoli gives further evidence of the triploid nature of this tissue.

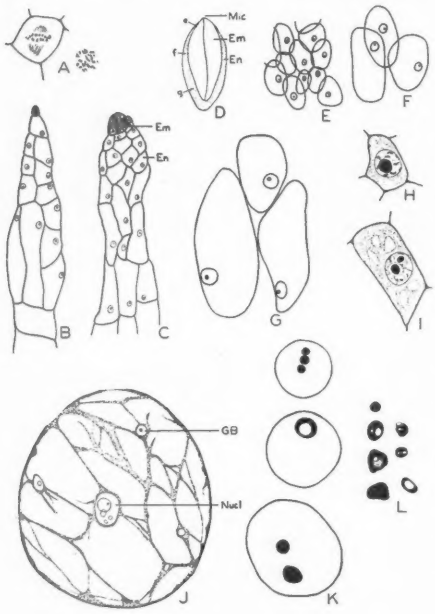


FIG. 4.—*Persea americana*. Endosperm. *A*, mitotic figure in endosperm. *B*, zygote stage of embryo and adjacent endosperm already in cellular stage. *C*, a proembryo stage with surrounding endosperm. *D*, diagram of embryo about 10 mm. long with endosperm. *E–G*, sketch of endosperm cells and nuclei at points *e*, *f*, and *g* of *D* showing relative sizes. *H*, *I*, endosperm cells from near base of embryo. *J*, large nucleus from endosperm tissue showing nature of peripheral chromatin threads, nucleoli, and general structure. *K*, three large endosperm nuclei with different numbers of nucleoli. *L*, types of nucleoli found in endosperm nuclei. *Em*, embryo; *En*, endosperm; *GB*, granular body; *Mic*, micropyle; *Nucl*, nucleolus.

The development of the endosperm precedes that of the embryo. In the earliest stages observed, cellular endosperm already surrounds the zygote or early proembryo (fig. 4*B*, *C*). Whether a free nuclear stage exists was not ascertained. As endosperm development continues, cell divisions occur only in the immediate vicinity of the embryo. The older cells are forced toward the chalazal end of the embryo sac and increase in volume. The isodiametric, meristematic endosperm cells adjacent to the embryo measure 30–60 $\mu$ in diameter and contain nuclei which range from 20 to 40 $\mu$ in diameter (fig. 4*H*, *I*). The ellipsoidal endosperm cells at the chalazal end may measure as much as 600 by 100 $\mu$ in length and breadth, and the extremely large nuclei in these cells range from 60 to 75 $\mu$ in diameter and in certain instances have exceeded 100 $\mu$ (fig. 4*G*). Reserve materials consist mainly of soluble sugars. No other ergastic substances such as oils, starch, or tannins were observed. When the tissue showed general signs of degeneration, oxalate crystals varying in size and form were noted in cells throughout.

The general appearance of the large resting or prophase nucleus of the endosperm tissue is that of a spherical, homogeneous mass covered on the surface by a thin network of chromatin material of variable thickness (fig. 4*J*). At certain intersections of these chromatin threads, granular bodies appear, and at others nucleoli are found. The central mass appears to be homogeneous throughout,

but occasionally strands of chromatin material appear to traverse the colorless matrix.

The nucleoli in endosperm cells vary in number from one to three (fig. 4*K*). The presence of three nucleoli supports the evidence that the endosperm is triploid tissue, since the number of nucleoli may indicate the number of chromosome complements in the nucleus (5). The nucleoli range in size from 4 to 26 $\mu$ in diameter and are generally spherical but may be elliptical or irregular in outline (fig. 4*L*). They are either homogeneous or may contain vacuoles of varying size and shape. The contents of the latter differ in refractive index.

The extremely large size of the endosperm nuclei makes them excellent objects for the study of nuclear morphology; they also may be of value in experiments in nuclear physiology.

EMBRYO DEVELOPMENT.—Development from the zygote (fig. 5*A*) to the multicellular proembryo (fig. 5*B–F*) takes place over a long period of time, probably many days. The earliest mitotic divisions of the proembryo have not been observed, but at the four-cell stage it is already spherical in form (fig. 5*B*). Thereafter, cell division continues in all planes. No suspensor is formed. As the proembryo increases in size, it remains spherical until it attains a diameter of about 0.2–0.3 mm., when cotyledonary differentiation is well marked (fig. 5*G*). The primordia of the cotyledons first appear as two small lobes at the chalazal end and elongate as narrow laminae of thin-walled parenchyma surrounded by an epidermis. The basal lobes of the cotyledons appear later (fig. 5*H*). At this stage the embryo measures about 1 mm. in length; the young radicle and plumule are distinguishable, and provascular tissue can be discerned. The cotyledonary node consists of a mass of meristematic cells distinguished from the surrounding parenchyma by their smaller size, deeply staining protoplasts, and relatively larger nuclei.

Differentiation of provascular tissue near the cotyledonary node and extending into the cotyledons is clearly defined

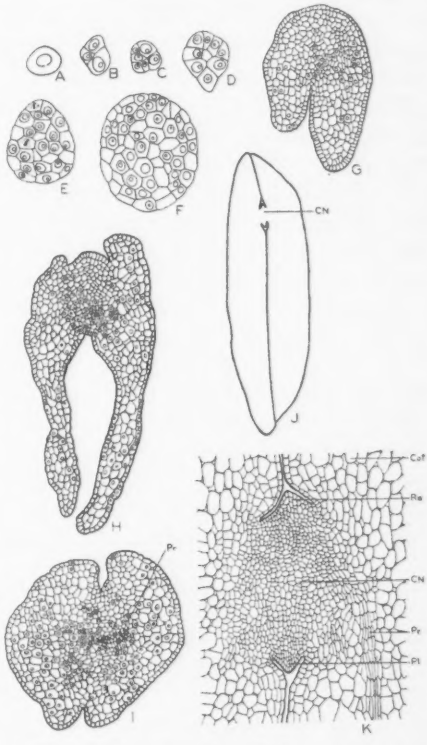


FIG. 5.—*Persea americana*. Embryo. *A–F*, successive stages of embryo development from zygote to multicellular, spherical proembryo. *G*, longisection through embryo about 0.5 mm. long showing early development of cotyledons. *H*, longisection through embryo about 1 mm. long showing cotyledons well differentiated and initiation of radicle. *I*, transection through cotyledonary node of 1-mm. embryo. *J*, later stage of embryo about 4 mm. long in radial section showing relative size of cotyledons, cotyledonary node, plumule, and radicle. *K*, detailed anatomy of cotyledonary node of 4-mm. embryo showing provascular tissue initiated in cotyledon. *CN*, cotyledonary node; *Cot*, cotyledon; *Pl*, plumule; *Pr*, provascular tissue; *Ra*, radicle.

in embryos about 3 mm. long (figs. 5*I–K*). Both lobes of the cotyledons are well developed in these embryos, having increased in length and thickness. Differentiation continues until in the mature embryo there are two hemispherical cotyledons with vascular strands located in a plane parallel to their flat faces and close to these surfaces.

## Summary

1. In the avocado, *Persea americana* Miller, flower parts develop in acropetal order. The flower is perfect, hypogynous, regular, and trimerous.

2. The ovule is anatropous and has two integuments. Megasporogenesis is initiated with the formation of a hypodermal archesporial cell which is distinguishable shortly after the integuments are evident. By successive divisions a linear row of four megaspores is formed, the chalazal cell functioning as the embryo sac. A normal type of development then follows which results in an eight-nucleate megagametophyte. The mature embryo sac is elongate and bulbous at the micropylar end. Cytological irregularities in the embryo sac, such as cross walls, multinucleate gametophytes, and a double embryo sac, are described.

3. Microsporogenesis begins with a hypodermal sporogenous cell. Primary sporogenous tissue gives rise to a central mass of pollen mother cells surrounded by a tapetal layer, the latter containing four nuclei in each cell. The pollen mother cells undergo meiosis and form tetrads which soon separate into individual spores. Mature pollen grains are spherical with no germ pores and are covered with short, conical spines.

4. The endosperm is a triple fusion tissue which becomes cellular and contains large, conspicuous nuclei. These nuclei sometimes attain diameters of 100 $\mu$.

5. During embryo development no suspensor is formed. Differentiation of cotyledons occurs when the spherical proembryo attains a diameter of about 0.2–0.3 mm.

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# AN ANATOMICAL STUDY OF ADVENTITIOUS ROOT DEVELOPMENT IN STEMS OF VACCINIUM CORYMBOSUM[1]

JOHN P. MAHLSTEDE AND DONALD P. WATSON

## Introduction

Many investigations concerned with the vegetative propagation of plants have been based upon the stimulation of adventitious roots by plant auxins. The stage of maturity of most plant parts governs partially the quantity of auxin present and consequently influences the ability to regenerate missing parts. To interpret adventitious root initiation clearly, it is necessary to understand the anatomical phases of development, whether one approaches these phenomena from the point of view of the influence of the auxin or of the influence of included tissue systems.

The present problem was designed to determine the anatomical aspects of root initiation and development in *Vaccinium corymbosum* L. An attempt to learn the origin of adventitious roots in various vascular plants has been made by certain investigators. VAN TIEGHEM and DOULIOT (5) reported that adventitious roots in young stems arise endogenously from the pericycle. They observed that semimature stem parts produce roots in the phloem parenchyma and that mature stem parts produce roots in the cambium region. VAN DER LEK (4) has reported that in older stems adventitious roots arise close to medullary rays and in association with secondary tissues. Apparently it is difficult to make generalizations concerning the exact tissue involved in the initiation of adventitious roots in stem cuttings. The origin varies with the stage of maturity of the shoot at the time of adventitious root development. PRIESTLY and SWINGLE (2) have described the change of the arrangement of tissue with age. With the development of the vascular cambium the ray cells in the cambial zone are compressed, and adventitious roots arise in association with the cambium. They reported that as growth continues the root initials arise in a group of undifferentiated air-free cells adjacent to the cambium.

Considerable emphasis has been placed upon stem anatomy and its relation to the capability of a woody cutting to produce adventitious roots. VAN DER LEK (4) concluded that the production of adventitious roots (morphological roots) near the base of the stem cuttings is restricted to certain morphological locations which were determined by the arrangement and type of tissues of the cutting.

## Material and methods

One-year-old stem cuttings of *V. corymbosum* L., var. Jersey, were collected in a dormant condition in March, 1949, from a 4-year-old planting growing in a peat bog north of Lansing, and from an established planting at the experiment station, South Haven, Michigan.

To insure an accurate comparison throughout these investigations, cuttings were of uniform diameter, each shoot providing not less than four cuttings. Cuttings were 4 inches long, their terminal horizontal cuts approximately $\frac{1}{8}$ inch

[1] Journal Article no. 1219 from the Michigan Agricultural Experiment Station.

above, and the basal diagonal cuts immediately below, a bud. All cuttings were placed in an airtight container into which had been introduced 0.01% propylene oxide (1). The cuttings were allowed to remain for a period of 2 hours, after which they were soaked in a solution of 7.2% slaked lime for a period of 10 minutes. The cuttings were rinsed in two changes of sterile distilled water. Two hundred and forty terminal and 240 basal cuttings were soaked in a sterile chamber in sterilized distilled water for a period of 10 minutes. All cuttings were "set" under aseptic conditions on July 9, 1949, in the conventional manner in a vertical position with the apex of each cutting approximately 1 inch above the surface of sterilized peat moss. Samples of five cuttings from each of the four lots were collected at 10-day intervals after the first 15 days had elapsed.

All cuttings were sectioned and prepared for microscopic observation in a similar manner. From the distal $1\frac{1}{4}$-inch section of each fresh stem part, transverse sections approximately 35 $\mu$ in thickness were cut. Four representative transverse sections and eight longitudinal sections were selected and transferred for 8 hours to a 3% solution of acetic acid containing orseillin BB (3). After rinsing in 40% alcohol, the material was stained for 10 minutes in a solution of aniline blue in 3% acetic acid. Washing and dehydrating with alcohol was followed by clearing in clove oil and xylol. Permanent mounting in Canada balsam was done in such a manner as to orient sections for future identification. The most desirable staining was characterized by a light purple color. Lignin, suberin, nuclei, and cytoplasm stained red (orseillin BB); cellulose stained blue (aniline blue). Water-soaked and dead tissue appeared yellow or brown.

## Observations

STEM ANATOMY.— Figure 1 is a drawing of a transverse section through a 1-year-old stem. The cuticular layer (*A*) on the surface covers a single layer of epidermal cells (*C*). Many other plants from a moist habitat are frequently found to have a thin cuticle, but the cuticle on the stem of this plant is thick. It is approximately the same depth as the subtending epidermal cells. The epidermis consists of a single layer of closely packed polyhedral cells, which on their exterior surfaces have blunt, saw-toothed apices. The cuticle is interrupted by stomata (*B*). The pores extend into the subtending cortical region. The guard cells are thin walled, but the wall is thicker on the side bordering the aperture. The cortical cells (*D*) are isodiametric. They are smaller and more closely packed toward the outer edge of the cortical region. The elliptical, to spherical, cortical cells subtending the stomatal mechanism are grouped more closely beneath the cavity and are somewhat smaller than those throughout most of the cortex. The outer cortical region varies from five to twelve cells in depth and forms a solid tissue between the epidermis and the air canals. Most cortical cells contain many chloroplasts. Centripetal to the outer cortical layer are large air ducts (*E*), usually oriented so that two ducts are adjacent to one another along the radial axis. In cross section these canals vary in thickness and are bounded by one and two rows of cortical parenchyma. Immediately inside the ring of air canals is a continuous band of thick-walled pericyclic fibers (*F*), each with a small lumen, ranging from three to five cells in depth. The phloem region (*G*) is not much if any greater in thickness than the adjacent pericyclic fiber region but is composed of smaller cells. The

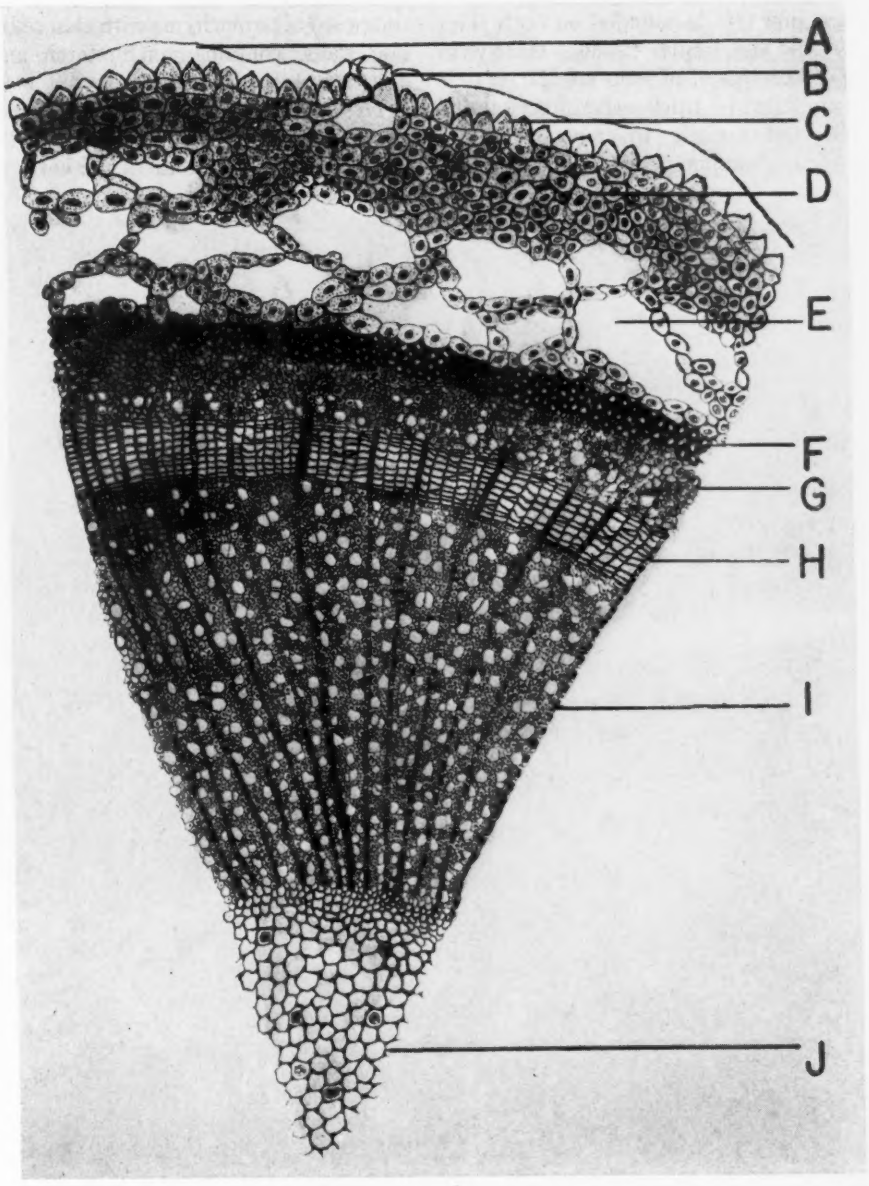


FIG. 1.—Segment of transverse section of 1-year-old stem of *Vaccinium corymbosum*. ×200. Cuticle (*A*), stoma (*B*), epidermal cell (*C*), cortex (*D*), air canals (*E*), pericyclic fibers (*F*), phloem region (*G*), cambium (*H*), xylem (*I*), pith (*J*).

cambium (*H*) is bounded on both sides by new and similar tissues. The xylem (*I*) is composed of long angular vessels surrounded by thick-walled fibers and is dissected radially by rows of thick-walled xylem parenchyma. The pith (*J*), composed of parenchyma with thin cellulose walls, contains much starch and many crystals.

DEVELOPMENT OF ROOTS.—Adventitious roots of hardwood cuttings arise endogenously at the base of the cutting.

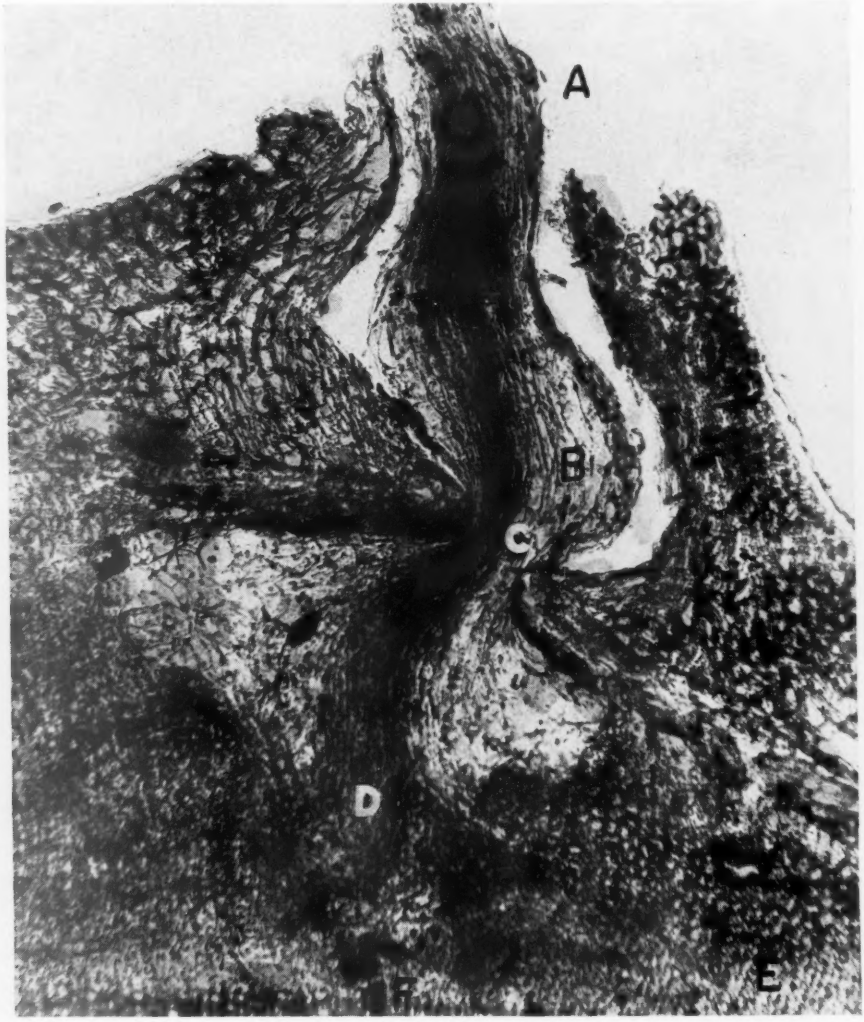


FIG. 2.—Transverse section through stem cutting showing bending of adventitious root beneath pericyclic fibers. ×91. Developing root (*A*); bending in cortex (*B*); root passing through pericyclic fibers (*C*); root origin in protophloem region (*D*); cambium region (*E*); xylem (*F*).

Microscopic examination of representative longitudinal and transverse sections from 480 stem cuttings showed that the root initials generally arise in the phloem region and, as described for other species (4), usually immediately adjacent to a xylem ray. At a very early stage no exact layer of cells delimits the root initial. Each cell of the group that is destined to become an initial is characterized by a large, centrally located nucleus and has a small vacuole. The first division of the cells in the primordium is periclinal without noticeable increase in cell size. Repeated periclinal and anticlinal divisions result in a primordium consisting of many small cells with a definite line of demarcation between the surrounding phloem elements. The young primordium enlarges as a result of increase in size and multiplication of included cells. It pushes toward the periphery of the stem, usually at right angles to the longitudinal axis. When the developing primordium does not progress radially, it follows either of two characteristic paths. The primordium either arises endogenously, enlarges, and bends prior to bursting through the pericylic fiber region (fig. 2) or the primordium arises endogenously, enlarges, and extends radially through the pericycle, where it may bend and discontinue development in the cortex beneath the epidermis and cuticle (fig. 3). Differentiation within the root initial was first observed as a solid region of xylem elements after the root primordium has pushed through the phloem and pericycle. There is no consistent evidence to show whether radial elongation of the

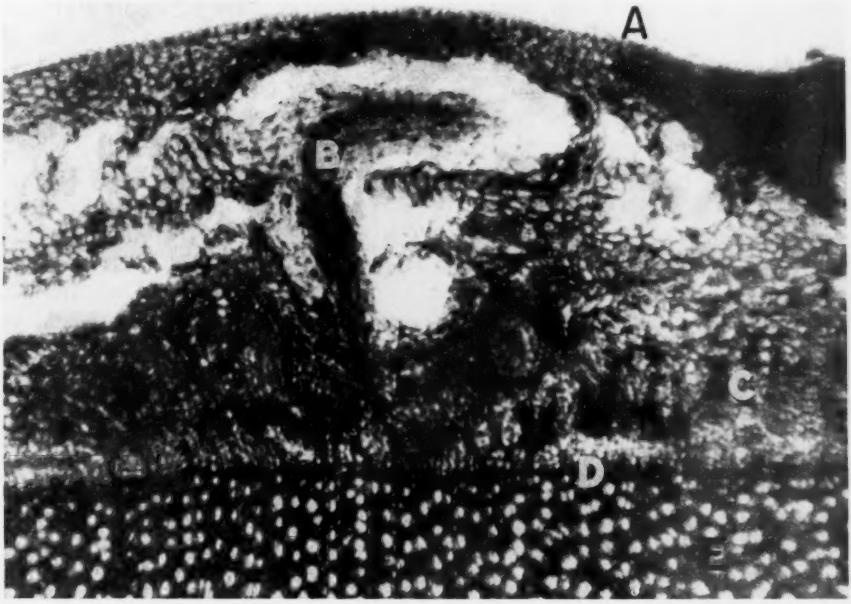


FIG. 3.—Transverse section through stem showing developing root bending beneath epidermis. ×79 Heavy cuticle (*A*); root bending in cortex beneath cuticle (*B*); phloem region (*C*); cambium (*D*); xylem (*E*).

developing primordium causes dissolution of, or mechanical separation of, adjacent tissue systems.

In blueberry cuttings there are two types of roots produced: those that develop as described located near the basal end of the cutting and those that emerge from callus tissue after the outer cells have become suberized. Adventitious roots were produced most commonly in association with a bud gap, although they did occur infrequently on an internode. At early stages they were visible to the naked eye as slight swellings.

MECHANICAL OBSTRUCTION TO ROOTING.—The ring of pericyclic fibers and the heavy cuticle are suggested as two characteristic, mechanical obstacles to the projection of the adventitious root. The photomicrograph of a transverse section showing a longitudinal section of a root primordium (fig. 2) illustrates the bending of the initial before it reaches the cortex. It is thought that this bending is produced as a result of the inability of the developing initial to break through the continuous ring of thick-walled pericyclic fibers. There is no evidence to indicate that the initial at this point dissolves the surrounding tissue in its path toward the outer regions of the stem. Contraction of the cell mass occurs in the cortical region of the new root as it passes through the fiber region; the root has a greater diameter both inside and outside of this region.

A curvature of the developing root directly beneath the epidermis at an angle as great as 90° is shown in figure 3. The radial projection of the developing root is halted as a result of the mechanical resistance offered by the thick cuticle and the densely packed cortical parenchyma. Only in severe cases does the developing primordium make a right-angle bend and discontinue further activity. Less severe cases have been observed where a root has bent slightly but subsequently forced its way through the cortex, epidermis, and cuticle, at some point far removed from the locus of a point directly radial to its origin.

If pericyclic fiber or cuticular restriction is the primary cause of obstruction, rooting percentages might be raised by using a weak acid or solvent to destroy or dissolve the cuticle, the epidermal layer, and even the pericyclic fibers, providing no damage was done to the phloem or cambium region where the initials originate.

## Summary

1. Hardwood cuttings of *Vaccinium corymbosum* L. were rooted under aseptic conditions to study the influence of the stem anatomy on adventitious root initiation and development. Description is given of the methods and techniques used.

2. Stem anatomy of the part used for propagation is discussed. Roots were found to originate in the cambium and phloem and to project through the callus or through the outer stele, cortex, and epidermis.

3. Descriptions are given of mechanical inhibition of root projection by the lignified pericyclic fibers and by the epidermis of closely packed cells with a thick cuticle.

4. It is suggested that, if some means could be provided to dissolve the cuticular and epidermal layers and the pericylic fibers, rooting percentages might be improved.

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# EFFECT OF INDOLEACETIC ACID ON ROOT GROWTH OF X-IRRADIATED PEAS

EDWARD S. MIKA

## Introduction

The effects of ionizing radiation upon living organisms have been studied extensively since the discovery of X-rays in 1895. In general, high doses of irradiation inhibit growth, and, as has been pointed out by many investigators (**2**), roots are especially radiosensitive. The effect on auxin and on its formation has been reported as the major factor in the inhibition of growth by ionizing radiation (**9**). The growth of nonirradiated roots in certain cases may be accelerated by low concentrations of indoleacetic acid (**10**). The present study is an attempt (*a*) to elucidate the nature of the inhibition of root growth by irradiation and (*b*) to determine whether the application of auxin in the form of indoleacetic acid (IAA) can counteract such inhibition.

## Material and methods

Since the effect of IAA upon an irradiated plant might be very small, it was essential to study individuals whose normal growth rate was relatively uniform. *Pisum sativum* L. var. Idaho White was selected as the most satisfactory of six different varieties of peas that were tested.[1] Dry seeds of a uniform diameter were obtained by sieving peas through apertures graduated in thirty-seconds of an inch. The modal class was used throughout the entire study.

Seed surfaces were sterilized by immersion for 10 minutes in 1% sodium hypochlorite. Preliminary experiments established that the peas could be soaked for 24 hours in running tap water with no apparent injury. Imbibed peas were irradiated, since moist peas are more radiosensitive (**3**, **5**).

Vermiculite (Zonolite Plaster Aggregate) was used as the substrate for germination. It was sterilized by either autoclaving at 15 pounds pressure for 30 minutes or by dry heat at 170° C. for 24 hours. The moisture content of the vermiculite favorable for growth was determined. HOAGLAND and BROYER'S nutrient solution (**6**) at 0.1 × and initially supplied at the rate of 2.5 ml/gm of oven-dry vermiculite yielded satisfactory results. This solution was added daily for replenishment at 200 ml/3500 gm of vermiculite. In all experiments peas were

[1] The following varieties were tested: Laxton's Progress, Perfectah, Alah, Mardelah, and Idaho White. Criteria for selection, in addition to uniformity, were: rapid growth rate, size, shape, viability, and ease of detection of axis in the dry seed. All peas were obtained through the courtesy of Wasburn and Wilson Seed Company, Moscow, Idaho.

grown in a dark chamber controlled at 25° C. and 85% relative humidity.[2]

The X-ray unit[3] was usually operated at 34 kilovolts and between 22 and 24 milliamperes. Dosage was 10 r per second in most experiments. Distance from the tube to the surface of the seeds was 10 inches. Since initial studies indicated that mass absorption was important in irradiation of peas, each pea was aligned so that the entire axis would be exposed to the X-ray beam. A circular Lucite disk, 6 inches in diameter and $\frac{1}{8}$ inch in thickness with 126 holes, each $\frac{1}{16}$ inch in diameter and in depth, held the peas in position during irradiation. Each pea was aligned after placement in its respective hole. A drop of water in the hole and a film of water over the upper surface of the disk retarded drying of the peas during irradiation. The disk was marked into sectors depending upon number of treatments being given and number of individuals per treatment. Sectoring was necessary, since a 3% variation in dose existed between individuals at the center and at the periphery of the disk. After alignment of the peas the disk was placed on a rotating turntable mounted directly below the beam, and irradiation took place. Each group, therefore, received relatively the same dosage as a result of alignment, sectoring, and rotation. For the most part irradiation was conducted at the same time each day.

Aqueous solutions of IAA were prepared according to the technique described by MACHT and GRUMBEIN (8). This procedure was used in all IAA treatments except in the 500 r+IAA experiment. Here potassium indoleacetate was used in the external solution. Lanolin paste was prepared by dissolving IAA crystals in a few drops of absolute alcohol, followed by incorporation of the alcoholic solution into lanolin. Approximately fifty thousand peas were grown in the entire study.

[2] American Instrument Company "Climatizer."

[3] The X-ray unit was made available through the courtesy of the Physics and Agronomy Departments, State College of Washington, Pullman, Washington.

## Experimentation

EFFECT OF X-RAY DOSAGE UPON GROWTH FROM IMBIBED PEA SEEDS OR OF PEA SEEDLINGS.—Imbibed peas were X-irradiated at 300, 3000, 6000, and 12,000 r and then germinated in the dark chamber for 5 days. During this interval the plants were removed daily from the substrate, measured, and then replanted in the original substrate. Between twenty-five and thirty plants were used in each treatment. Two responses occurred depending upon the dosage applied. Peas given 300 r showed irregular growth (fig. 1). The shoot growth was greater than that of the controls except for the interval between 48 and 96 hours after irradiation. The inhibition of root growth by 300 r was more pronounced than that of the shoots. Root growth of plants irradiated at the three higher doses had ceased at 120 hours, whereas the shoot growth still continued.

Another experiment was conducted to determine the effect of X-ray dosage between 500 r and 3000 r upon pea seedlings instead of imbibed peas. When the radicles of the seedlings were approximately 40 mm. long, uniform groups of fifteen plants each were irradiated at 500, 1000, 2000, and 3000 r, respectively, except for a nonirradiated control group. After irradiation the seedlings were grown in continuously aerated nutrient solution in the dark chamber.

Roots of seedlings receiving 1000, 2000, and 3000 r showed essentially the same sigmoid pattern of response (fig. 2).

The over-all difference in response to these dosages was relatively small, which suggests that the biological effectiveness of 1000 r in limiting root growth was almost as great as that of 3000 r. Irradiation at 500 r, however, was clearly less effective than the other three doses.

TIONS UPON GROWTH FROM IMBIBED PEAS X-IRRADIATED AT 3000 R.—Root growth may be accelerated or inhibited depending upon the concentration of IAA applied; concentrations of $10^{-9}$ M IAA have been reported as inducing optimal root growth (10). An investigation was

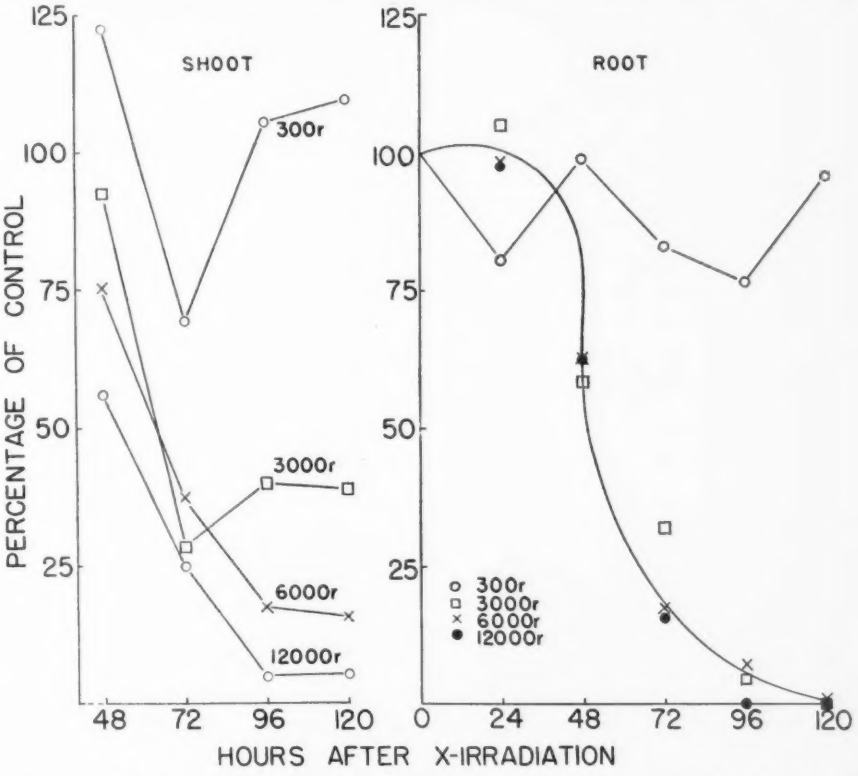


FIG. 1.—Effect of various dosages of X-irradiation upon growth from imbibed peas. *Left*, shoot growth. *Right*, root growth.

It seems that seedlings were more radiosensitive than imbibed seeds. When seedlings were irradiated at 500 r, a 50% inhibition of root growth occurred within 40 hours after treatment (fig. 2). When imbibed seeds were irradiated at 3000 r, a 50% inhibition of root growth had taken place 52 hours later (fig. 1).

EFFECT OF VARIOUS IAA CONCENTRA-

conducted to determine (*a*) whether root systems of irradiated plants would respond to IAA, (*b*) the magnitude of the response, and (*c*) what concentration would bring about optimal growth of the roots. Imbibed peas were irradiated at 3000 r and germinated in vermiculite as earlier described except that the nutrient solution also contained different concen-

trations of IAA. The peas were removed at intervals, measured, and replaced. Between eighteen and thirty-seven peas were grown in each group.

Root systems of imbibed peas treated with 3000 r not only responded to IAA but also showed a differential response to various concentrations (fig. 3) similar to that reported for normal plants. The optimal concentration of IAA for root growth lay between $10^{-8}$ and $10^{-9}$ *M*. The inhibition of growth at $10^{-5}$ *M* was clearly evident but was less apparent at $10^{-7}$ and $10^{-6}$ *M* IAA. Thus, although root growth was already somewhat inhibited by X-irradiation, IAA inhibited growth even further when applied at high concentrations.

EFFECT OF $10^{-9}$ *M* IAA ON ROOT GROWTH FROM IMBIBED PEAS X-IRRADIATED AT 3000 R.—SKOOG (9) concluded from irradiation studies on *Avena* coleoptiles and *Helianthus* hypocotyls that the capacity for growth is unimpaired by X-irradiation. He reported that the increase in growth resulting from the application of auxin is as great as the decrease in growth caused by irradiation. Thus it seems that a complete counteraction of the effect of irradiation can occur under certain conditions. X-irradiated peas were studied, therefore, to ascertain whether and to what extent the inhibitive effect of X-irradiation on their root growth could be counteracted by an exogenous supply of IAA ($10^{-9}$ *M*) either

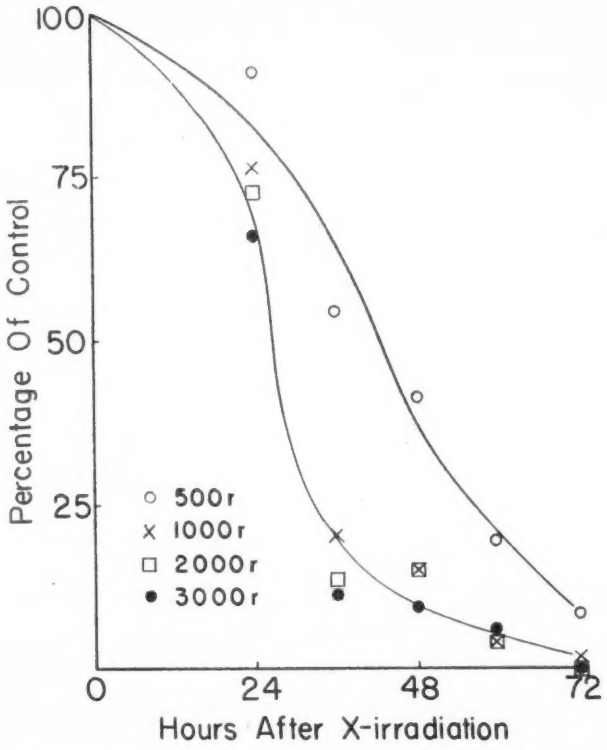


FIG. 2.—Effect of various dosages of X-irradiation upon root growth of pea seedlings

before or after the irradiation. The design of the experiment appears from table 1.

All peas were soaked in aerated nutrient solution for 18 hours prior to X-irradiation and then placed in vermiculite containing the dilute nutrient. Two IAA and grown for 72 hours. The peas were removed daily from the substrate, measured, and replaced. Two hundred milliliters of fresh IAA and/or nutrient solution were added daily to the substrate, depending upon the treatment. It

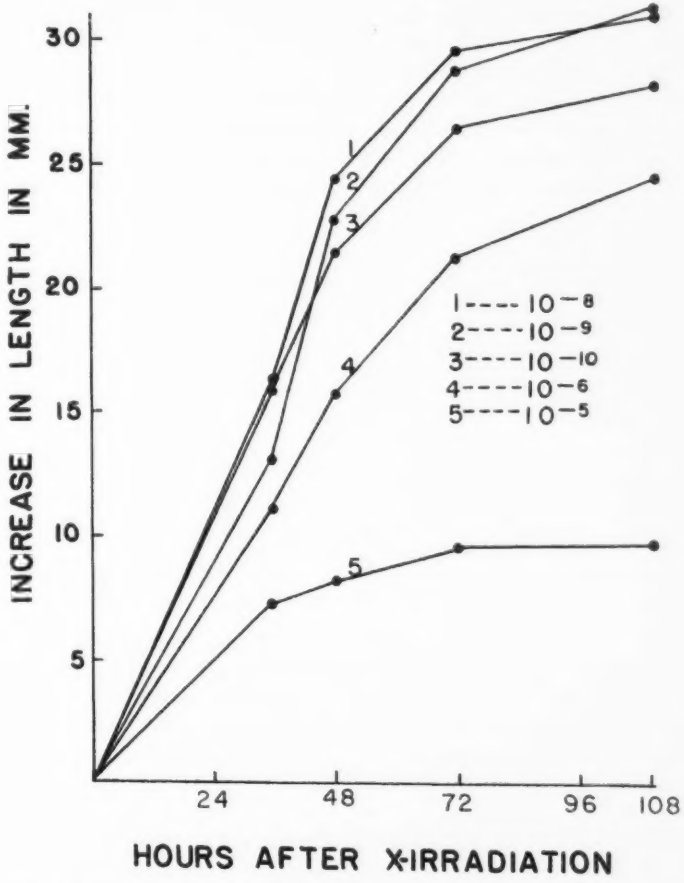


FIG. 3.—Effect of different concentrations of IAA upon root growth from imbibed peas X-irradiated at 3000 r. Effect at $10^{-7}$ *M* IAA, which was similar to effect at $10^{-10}$ *M* IAA, was omitted to facilitate presentation.

groups were treated with $10^{-9}$ *M* IAA prior to irradiation—group 4 for 18 hours and group 5 for 36 hours. After irradiation, all groups were transferred to a vermiculite substrate with or without

is apparent that IAA did not counteract the inhibitive effect of irradiation (table 1). In fact, after 48 hours the growth rate of irradiated plants was greater in the absence of IAA.

EFFECT OF IAA ON GROWTH OF PEA SEEDLINGS X-IRRADIATED AT 500 R.—Peas were germinated and grown as previously described. When roots were approximately 60 mm. long, the seedlings were selected for uniformity and irradiated at 500 r. Auxin was applied in two different ways after irradiation: (*a*) intact plants were grown in a nutrient solution containing $10^{-9}$ *M* potassium indoleacetate and (*b*) the cut surface of plants whose shoots were decapitated was smeared with a lanolin paste containing 0.2% IAA immediately following X-irradiation. All solutions and pastes were renewed daily. Daily decapitations were also done. Eight groups of fifteen plants each were used in the study.

The growth rate of roots was less inhibited by irradiation when IAA was applied to irradiated seedlings (fig. 4). The growth of roots of irradiated, intact, IAA-treated plants for the period between 24 and 48 hours after X-irradiation was approximately 22% greater than that of irradiated, intact plants not receiving IAA. This small difference is statistically significant. During the same period root growth of irradiated, decapitated, IAA-treated seedlings was 12% greater than that of irradiated, decapitated seedlings without exogenous IAA. Thus, the lessened effect of irradiation which resulted from treatment of irradiated plants with IAA is manifested irrespective of the mode of entry of the applied IAA. Abnormal branching similar to the type described by JOHNSON (7) occurred in a few individuals of the irradiated, intact, non-IAA treated group.

A correlation between auxin and the development of the lateral buds has already been pointed out (9). In the normal plant the terminal bud controls the growth of the lateral buds to a large degree. If the terminal bud is removed, the development of the bud in the axil of the cotyledon is promoted. All nonirradiated, decapitated plants and 40% of the irradiated, decapitated plants formed secondary shoots in the absence of IAA. In the presence of IAA, shoots were not developed in decapitated seedlings regardless of whether they were X-irradiated or not. Initiation and development of lateral roots in intact and decapitated plants treated with IAA 72 hours after irradiation was greater than in their respective controls: intact, nonirradiated seedlings produced 200 and 163 lateral roots in the presence and absence of IAA, respectively, whereas intact, irradiated

TABLE 1

EFFECT OF $10^{-9}$ MOLAR IAA ON DAILY AVERAGE ROOT GROWTH (MM.) FROM IMBIBED PEAS X-IRRADIATED AT 3000 R (VALUES BASED ON 22–31 PLANTS)

| GROUP | TREATMENT | | | | DAILY GROWTH | | |
|---|---|---|---|---|---|---|---|
| | Before X-ray | | X-ray | After X-ray | 24 hours after irradiation | 48 hours after irradiation | 72 hours after irradiation |
| | 0–18 hours | 18–36 hours | | Substrate | | | |
| 1 | No IAA | Vermiculite | None | Vermiculite | 16.6 | 31.4 | 31.6 |
| 2 | No IAA | Vermiculite | 3000 r | Vermiculite | 9.8 | 3.8 | 4.1 |
| 3 | No IAA | Vermiculite | 3000 r | Vermiculite+IAA | 9.7 | 4.7 | 1.1 |
| 4 | No IAA | Vermiculite+IAA | 3000 r | Vermiculite+IAA | 9.9 | 3.7 | 0.7 |
| 5 | IAA added | Vermiculite+IAA | 3000 r | Vermiculite+IAA | 9.2 | 3.1 | 0.5 |

seedlings produced 11 and 5 lateral roots under the respective conditions. Decapitated, nonirradiated plants produced 258 and 109 lateral roots in the presence and absence of IAA, respectively, whereas decapitated, irradiated plants produced 37 and 5 lateral roots. Dry weight of roots of decapitated, nonirradiated plants treated with IAA was 306 mg., while the weight of roots of plants not treated with IAA was 219 mg.

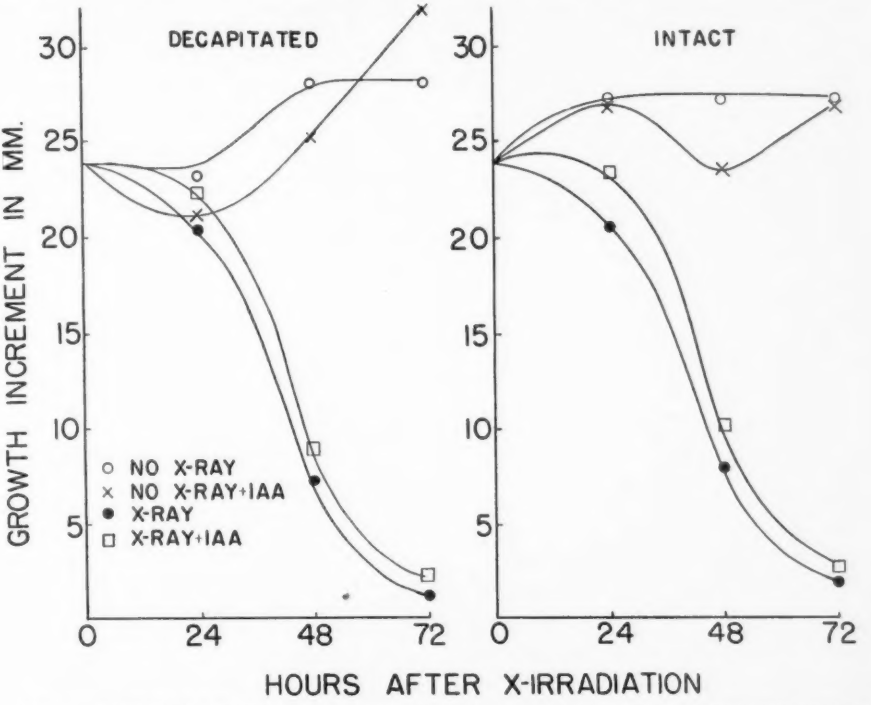


FIG. 4.—Effect of IAA on root growth of pea seedlings X-irradiated at 500 r. *Left*, decapitated seedlings with 2 mg IAA/gm lanolin applied to stump. *Right*, intact seedlings with $10^{-9}$ *M* IAA in nutrient solution.

## Discussion

The effect of added IAA in lessening the inhibition in X-irradiated pea seedlings indicates that inactivation of IAA may be one of the factors involved in the inhibition of growth by X-irradiation but does not seem to be the major factor as stated by SKOOG (9). His studies showed that the decrease in growth rate resulting from X-irradiation of *Avena* coleoptiles and *Helianthus* hypocotyls may be counteracted to a large degree by the application of IAA. Only a small counteraction of the effect of X-irradiation was obtained in the present study when pea seedlings were irradiated at 500 r, and IAA was applied. One possible explanation for the discrepancy between the studies, in addition to the different species of plants used and the dosage applied, may be that the magnitude of inhibition of growth was not the same in the two investigations. SKOOG pointed out that "in decapitated sections not sup-

plied with auxin, the growth is so small that a difference between the control and irradiated plants can not be determined with certainty." In the present study, inhibition resulting from X-irradiation was marked in both intact and decapitated plants as compared with their respective nonirradiated controls. At low dosages of X-irradiation the inactivation of free acid auxin is proportional to dosage (4). It seems, however, that, beyond a given dosage of X-irradiation, complete restoration of growth may not occur. BARRON (1) reported that the extent of reactivation is related to the extent of inactivation. When phosphoglyceraldehyde dehydrogenase was inhibited 21% at 100 r *in vitro*, complete reactivation of the enzyme occurred upon addition of glutathione; at 50% inhibition at 200 r, 62% reactivation was obtained; and at 94% inhibition at 500 r, only 10% reactivation occurred.

Root length, the criterion for growth in this study, results from cell division and cell elongation. The concentrations of IAA, promoting cell division and cell elongation respectively, are different (10). In this study when irradiated plants were treated with IAA, an increase in the initiation of roots and an increase in root length both resulted. A cyto-histological investigation is necessary for further elucidation of the effect of IAA on the growth and development of X-irradiated tissues.


## Summary

1. Idaho White peas (*Pisum sativum* L.) were treated with different dosages of X-irradiation (34 kv. and 22–24 ma.), and the inhibiting effect upon growth was studied.

2. Imbibed, irradiated peas responded to IAA which was applied in an attempt to counteract the inhibition of root growth caused by X-irradiation. The optimal concentration of added IAA for the root growth of peas irradiated at 3000 r lay between $10^{-8}$ and $10^{-9}$ *M*.

3. A partial reduction in the amount of inhibition of growth was obtained when intact and decapitated seedlings, irradiated at 500 r, were treated with $10^{-9}$ *M* IAA and with 0.2% IAA in lanolin paste, respectively.

4. The development of the axillary bud in decapitated seedlings, irrespective of irradiation, was promoted in the absence, and inhibited in the presence, of IAA.

5. The initiation and development of lateral roots was accelerated by IAA, regardless of irradiation.


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# OCCURRENCE OF ANTIMICROBIAL SUBSTANCES IN CHLOROPHYLLOSE PLANTS GROWING IN FLORIDA

GRACE C. MADSEN AND ANNE L. PATES

## Introduction

Interest in the possibility that important naturally occurring antimicrobial substances may be discovered in common green plants has been increasing. The first systematic search for such substances was carried out by OSBORN at the University of Oxford. The results, published in 1943 (**17**), indicated that extracts of Ranunculaceae showed a greater inhibiting effect than those of any other family of plants studied. OSBORN also concluded that well-known drug plants, such as *Atropa* and *Datura*, sources of atropine or belladonna, and *Digitalis*, tested by his method show no inhibitory power.

In this country investigations have been conducted in Michigan, Indiana, Ohio, Vermont, and elsewhere. LUCAS and LEWIS (**16**) tested for antibacterial substances in the organs of higher plants in Michigan. The plant which showed most promise in their survey was *Lonicera tatarica* (Tatarian honeysuckle). It contained two active principles, one effective against *Staphylococcus aureus* and the other against *Escherichia coli*. HUDDLESON *et al.* (**13**) used twenty-three genera in Michigan and found activity in six belonging to six different families. The most active substances were found in *Allium* (garlic and onion), *Rheum* (rhubarb), and *Ribes* (a white currant). SAUNDERS *et al.* (**18**) investigated plants growing in Indiana, and, although about a tenth of the specimens showed some degree of inhibitory activity, no one sample was encountered with exceptionally high antimicrobial properties. HAYES (**12**) used aqueous extracts of 231 species of higher plants common to Ohio in testing for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Erwinia carotovora*, and *Phytomonas tumefaciens*. Of the species tested, 46 inhibited growth of one or more test organisms, and 18 of these showed marked inhibitory properties. CARLSON *et al.* (**6**) in Ohio showed that approximately a fifth of the plant extracts studied in their survey were bacteriostatic. LITTLE and GRUBAUGH (**15**) tested common garden plants in Vermont and found that beans and corn gave best results against human pathogens.

As far as is known to the authors, no one in the course of a search for antimicrobial substances in chlorophyllose plants has reported work of this type using plants in southeastern United States. Accordingly, this project was undertaken at Florida State University in August, 1950.

## Material and methods

The plants tested were mostly green seed-plants, some pteridophytes, a few bryophytes, and an alga, all selected at random to secure wide variety. Most specimens were prepared for testing immediately upon collecting. In general, leaves, roots, stems, fruits, and seeds were extracted separately. Preparation consisted of cutting up 50 gm. of fresh material with scissors and then macerating it in a Waring Blendor with 50 ml. of distilled water. Four 20-gm. samples of macerate were then weighed out and placed in four bottles, each containing, respectively, 20 ml. of one of the following solvents: 1.5% $H_2SO_4$, 1.5% NaOH, a $H_3BO_3$-NaOH buffer solution of pH 9.0, or distilled water. The four lots were then refrigerated for an 18–24-hour extraction period, after which the pH of the solutions was determined. For those extracts testing below pH 4.5 or above 8.0 the pH was then adjusted with a phosphate buffer to between 6.4 and 7.8 to avoid inhibitory effects due to high alkalinity or acidity.

Many types of test organisms have been used in antimicrobial surveys. The micro-organisms employed in the present survey were selected on the following bases: (*a*) an organism which would give results comparable with the results obtained by others and (*b*) organisms against which there are few or no antibiotics available. The following three organisms were selected:

*Staphylococcus aureus* (P209): a gram-positive organism which is most frequently used as a standard in antibiotic work.

*Pseudomonas aeruginosa:* a gram-negative, motile rod which develops a green pigment and is the cause of so-called "blue-green pus" in infected wounds. It also may give rise to chronic infections which are difficult to treat.

*Candida albicans:* a pathogenic yeast which may cause a variety of infections ranging from conditions affecting the external skin to deep-seated infections of the respiratory and digestive tract.

The method of determining whether inhibiting substances were present in plant extracts was as follows: Nutrient agar seeded with an 18–24-hour culture of *S. aureus*, *P. aeruginosa*, or *C. albicans* was poured into sterile Petri dishes and allowed to harden. Sterile filter-paper disks were placed on the inoculated plates, and 0.1 ml. of prepared extract from one of the four lots for each plant part was dropped onto each disk. After being incubated for 24 hours at 37° C. the plates were read for zones of inhibition, and the width of these zones was recorded in millimeters.

## Results

Table 1 records the effectiveness of the plant extracts in inhibiting *S. aureus*, *P. aeruginosa*, and *C. albicans*. The 126 plant parts tested comprised 102 species which represented 95 genera and 65 families (**1, 2, 4, 7, 8, 10, 11, 14, 19, 20**). Over 1500 tests were run and recorded.

Of the 102 species, 58 showed inhibitory power. These included 1 Phaeophyta, 4 of 6 tested Bryophyta, and 53 of 95 tested Tracheophyta. Most of the vascular plants were angiosperms, of which 46 of 83 showed activity. Thirty-nine of 72 dicots and 8 of 11 monocots were inhibitory. Of the 102 species tested, 17 inhibited the growth of *S. aureus*, 15 inhibited the growth of *P. aeruginosa*, and 49 inhibited the growth of *C. albicans*.

## Discussion

The method employed for testing the extracts of this survey was considered to be only a qualitative one showing the

TABLE 1

ACTIVITY OF PLANT EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS (SA), PSEUDOMONAS AERUGINOSA (PA), AND CANDIDA ALBICANS (CA)

Plant part: E, entire plant; Fl, flower; Fr, fruit; L, leaf; P, petiole; R, root; Rh, rhizome; S, seed; St, stem. Inhibition: P, partial; C, complete. Zone of inhibition: 1 = 10–20 mm., 2 = 20–30 mm., 3 = 30–40 mm., 4 = 40–60 mm., 5 = 60–90 mm.

| PLANTS | PLANT PART | INHIBITION OF PLANT EXTRACTS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5% $H_2SO_4$ | | | 1.5% NaOH | | | pH 9.0 Buffer | | | $H_2O$ | | |
| | | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca |
| Fucaceae | | | | | | | | | | | | | |
| *Sargassum natans* (L.) Meyen | E | | | | | | | | | C1 | | | |
| Marchantiaceae | | | | | | | | | | | | | |
| *Conocephalum conicum* (L.) Dum | E | | | | | | C2 | | | C2 | | | P2 |
| *Dumortiera hirsuta* (Sw.) Nees | E | | | | | | | | | C1 | | | |
| *Marchantia domingensis* L. and L | E | | | | | | | | | | | | |
| Leskeaceae | | | | | | | | | | | | | |
| *Thuidium delicatulum* (Hedw.) Mitt. | E | | | | | | | | | | | | |
| Sphagnaceae | | | | | | | | | | | | | |
| *Sphagnum portoricense* Hampe | E | | | | C1 | C1 | P2 | | | C1 | | | P2 |
| *S. strictum* Sull | E | | | | | | | | | C2 | | | |
| Lycopodiaceae | | | | | | | | | | | | | |
| *Lycopodium alopecuroides* L | E | C2 | C2 | P1 | | | P2 | | | | | | |
| *L. cernuum* L | E | | | | | | | | | C1 | | | |
| Selaginellaceae | | | | | | | | | | | | | |
| *Selaginella ludoviciana* A. Br | E | | | | | | C3 | | | | | | |
| Polypodiaceae | | | | | | | | | | | | | |
| *Adiantum capillus-veneris* L | L | | | | | | | | | | | | |
| *Onoclea sensibilis* L | E | | | | | | | | | | | | |
| Ginkgoaceae | | | | | | | | | | | | | |
| **Ginkgo biloba* L | L | | | | | | | | | C2 | | | |
| Pinaceae | | | | | | | | | | | | | |
| **Cedrus deodara* Loud | L | | | | | | | | | | | | |
| *Pinus palustris* Mill | St | | | | | | | | | | | | |
| *Taxodium ascendens* Brongn | L, St | | | | | | P1 | | | P1 | | | |
| Juniperaceae | | | | | | | | | | | | | |
| *Sabina silicicola* Small | L, St | | | | C1 | | | | | P1 | | | |
| Taxaceae | | | | | | | | | | | | | |
| **Podocarpus macrophylla* (Maki) Endl | L, Fr | | | | | | | | | | | | |
| | St | | | | | | | | | | | | |
| *Tumion taxifolium* (Arn.) Greene | L | | | | | | | | | C2 | | | |
| Aceraceae | | | | | | | | | | | | | |
| *Acer saccharinum* L | L | | | | | | C2 | | | | | | |
| Alpiniaceae | | | | | | | | | | | | | |
| **Hedychium coronarium* Koenig | L | | P1 | | | | | | | C2 | | | |
| Ammiaceae | | | | | | | | | | | | | |
| *Sanicula gregaria* Bicknell | L | | | | | | | | | | | | |
| Amygdalaceae | | | | | | | | | | | | | |
| *Padus virginiana* (L.) Mill | L | | | | | | P2 | | | P2 | | | |
| Anacardiaceae | | | | | | | | | | | | | |
| *Rhus copallina* L | L | | | | | P2 | C5 | | | C2 | | | |
| *R. toxicodendron* L | L | | | | | | | | | | | | |
| Annonaceae | | | | | | | | | | | | | |
| *Asimina parviflora* (Michx.) Dunal | L | | | | | | | | | | | | |
| Apocynaceae | | | | | | | | | | | | | |
| **Trachelospermum jasminoides* Lem. | L, St | | | | | | | | | C2 | | | |
| Araceae | | | | | | | | | | | | | |
| **Xanthosoma* sp | P | C3 | C1 | | | P1 | | | | | | | |

* Cultivated plants.

TABLE 1—*Continued*

| Plants | Plant part | Inhibition of plant extracts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5% $H_2SO_4$ | | | 1.5% NaOH | | | pH 9.0 Buffer | | | $H_2O$ | | |
| | | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca |
| Araliaceae | | | | | | | | | | | | | |
| **Hedera canariensis* Willd. | L | | | | | | C1 | C1 | | C2 | | | |
| Arecaceae | | | | | | | | | | | | | |
| *Serenoa repens* (Bartr.) Small | L | | | | | | P1 | | | P1 | | | |
| Asclepiadaceae | | | | | | | | | | | | | |
| *Asclepias tuberosa* L. | L, St | | | | | | | | | | | | |
| | T | | | | | | | | | | | | |
| Berberidaceae | | | | | | | | | | | | | |
| **Nandina domestica* Thunb. | L | | | | | | | | | P1 | | | |
| Bignoniaceae | | | | | | | | | | | | | |
| *Bignonia radicans* L. | L | | | | | C1 | | | | | | | |
| **Clytostoma callistegioides* Bur. | L | | C1 | C1 | | | | | | | | | C2 |
| Bromeliaceae | | | | | | | | | | | | | |
| *Dendropogon usneoides* Ref. | E | | | | | | | | C1 | | | | |
| Buettneriaceae | | | | | | | | | | | | | |
| **Firmiana platanifolia* (L.) R. Br. | L | | | | | | | | | P1 | | | |
| | S | | | | | | | | | | | | |
| Caprifoliaceae | | | | | | | | | | | | | |
| **Abelia grandiflora* Rehd. | L, St | | | | | | | | | C2 | | | |
| *Sambucus simpsonii* Rehder | L | | | | | | P4 | | | P1 | | | P1 |
| Carduaceae | | | | | | | | | | | | | |
| *Baccharis halimifolia* L. | L | | | | | | C5 | | | C5 | | | C5 |
| *Conoclinium coelestinum* (L.) DC. | L, St | | | | | | | | | | | | |
| *Eupatorium capillifolium* (Lam.) Small | L, St | | | | | | | | | C1 | | | |
| *E. perfoliatum* L. | L, St | | | | | | | | | P1 | | | |
| *Liatris chapmanii* (T. and G.) Kuntze | L, St | | | | | | | | | P1 | | | |
| *Solidago* sp. | L | | | | | | | | | | | | |
| | Fl | | | | P1 | | | | | | | | P1 |
| Chenopodiaceae | | | | | | | | | | | | | |
| *Atriplex arenaria* Nutt. | L, St | | | | | | | | | P1 | | | |
| Compositae | | | | | | | | | | | | | |
| **Dahlia* sp. | L | | | | | | | | | | | | |
| Convolvulaceae | | | | | | | | | | | | | |
| **Ipomoea purpurea* Lam. | L | | | | | | | C1 | | | | | |
| | St | | | | | C1 | | C2 | | | | | |
| Cornaceae | | | | | | | | | | | | | |
| *Cornus florida* L. | L | P1 | | | | | P1 | P1 | | C1 | P1 | | C2 |
| Dioscoreaceae | | | | | | | | | | | | | |
| **Dioscorea bulbifera* L. | L, T | | | | | | | | | C2 | | | |
| Droseraceae | | | | | | | | | | | | | |
| *Drosera tracyi* Macfarlane | L, St | | | | | C1 | C2 | | | C2 | | | |
| Ebenaceae | | | | | | | | | | | | | |
| *Diospyros virginiana* L. | L | | P1 | | | | | | | | | | |
| Empetraceae | | | | | | | | | | | | | |
| *Ceratiola ericoides* Michx. | L | | | | C1 | | C1 | | | | | | |
| Epilobiaceae | | | | | | | | | | | | | |
| *Jussiaea erecta* L.† | L, St | | | | | | | | | P1 | | | P1 |
| *J. leptocarpa* Nutt.† | L, St | | | P1 | | | P2 | P1 | | C1 | P1 | | P1 |
| Euphorbiaceae | | | | | | | | | | | | | |
| **Aleurites fordii* Hemsl. | L | | P1 | | | P1 | | | | P1 | P1 | | P1 |
| | St | | P1 | | | P1 | | | | | | | |
| | Fr | | | | | | | | | C1 | | | C1 |
| *Euphorbia pulcherrima* Willd. | L | | | C5 | | | C5 | | | C5 | | | C5 |
| *Ricinus communis* L. | L | | | | | | | | | C2 | | | |

† Species tentative.

TABLE 1—*Continued*

| Plants | Plant part | Inhibition of plant extracts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5% $H_2SO_4$ | | | 1.5% NaOH | | | pH 9.0 Buffer | | | $H_2O$ | | |
| | | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca |
| Fabaceae | | | | | | | | | | | | | |
| *Crotalaria retzii* A. Hitchc. | L, St | | | | | | | | | | | | |
| | S, Fr | | | | | | | C1 | | | C1 | | |
| *Erythrina herbacea* L. | L | | | | | | | | | | | | |
| *Pueraria thunbergina* (S. and Z.) Benth. | L | | | | | | | | | | | | P2 |
| Fagaceae | | | | | | | | | | | | | |
| *Quercus marilandica* Muench. | L | P1 | | P1 | | | P1 | P1 | | P2 | P1 | | P2 |
| Hydrangeaceae | | | | | | | | | | | | | |
| *Hydrangea quercifolia* Bartr. | L | | | | | | P1 | C1 | | C2 | | | |
| Hydroleaceae | | | | | | | | | | | | | |
| *Nama corymbosum* (Macbride) Kuntze | L, St | | | | | | | | | | | | |
| *N. quadrivalve* (Walt.) Kuntze | L | C2 | C2 | | | | | | | | | | |
| | Rh | | | | | | | | | P1 | | | P1 |
| Iridaceae | | | | | | | | | | | | | |
| **Gladiolus* sp. | L | | | | | | | | | | | | |
| Juglandaceae | | | | | | | | | | | | | |
| *Hicoria pecan* (Marsh) Britton | L | | | | | | P2 | | | P2 | | | P1 |
| *Juglans nigra* L. | L | | | | | | | | | C2 | | | C1 |
| Lamiaceae | | | | | | | | | | | | | |
| *Clinopodium coccineum* (Nutt.) Kuntze | L, St | | | | | | P2 | | | C1 | | | P2 |
| Lauraceae | | | | | | | | | | | | | |
| **Cinnamomum camphora* Nees and Eberm. | L | | | | | | | | | | | | |
| *Sassafras variifolium* (Salisb.) Ktze. | L | | | P3 | | | | | | | | | |
| Leguminosae | | | | | | | | | | | | | |
| **Glycine max* Merr. | L, Fr | | | | | | | | | | | | |
| Liliaceae | | | | | | | | | | | | | |
| **Asparagus spengeri* Regel. | St | | | | | | | | | | | | C2 |
| Lythraceae | | | | | | | | | | | | | |
| *Decodon verticillatus* Ell. | L, St | | | | | | | | | C1 | | | P1 |
| Magnoliaceae | | | | | | | | | | | | | |
| *Illicium parviflorum* Michx. | L | | | | | | | | | | | | |
| *Liriodendron tulipifera* L. | L | | | | | | | C1 | | | C1 | | |
| Malvaceae | | | | | | | | | | | | | |
| **Hibiscus mutabilis* L. | L | C1 | P1 | P2 | C1 | | C2 | C1 | P1 | | C1 | P1 | C2 |
| | S, Fr | C1 | P1 | | C2 | P1 | C5 | C1 | | C5 | C1 | P1 | C5 |
| **Malvaviscus grandiflorus* Hort. | L | | | | | | | | | | | | C1 |
| Mimosaceae | | | | | | | | | | | | | |
| **Albizzia julibrissin* Durazz. | L | | | | | | | | | C1 | | | |
| | Fr | | | | | | P1 | | | C2 | | | P2 |
| Nelumbonaceae | | | | | | | | | | | | | |
| *Nelumbo lutea* (Willd.) Pers. | L | | | | | C1 | P1 | | | | | | |
| | P | | | | | | C3 | | | | | | P5 |
| Palmaceae | | | | | | | | | | | | | |
| **Butia capitata* Becc. | L | | | | | | P1 | | | P1 | | | |
| Pittosporaceae | | | | | | | | | | | | | |
| **Pittosporum tobira* Ait. | L, St | | | | | | | | | | | | |
| | Fr | | | | | | | | | | | | |
| Polygonaceae | | | | | | | | | | | | | |
| **Antigonon leptopus* Hook. and Arn. | L | | | | | | C1 | P1 | | C2 | | | |
| *Thysanella fimbriata* (Ell.) A. Gray | L | | | | | | | | | C1 | | | |

TABLE 1—*Continued*

| Plants | Plant part | Inhibition of plant extracts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.5% $H_2SO_4$ | | | 1.5% NaOH | | | pH 9.0 Buffer | | | $H_2O$ | | |
| | | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca | Sa | Pa | Ca |
| Pontederiaceae | | | | | | | | | | | | | |
| *Eichornia crassipes* (Mart.) Solms.. | L | .... | C1 | P1 | .... | .... | .... | C1 | .... | C1 | .... | .... | .... |
| | St | .... | .... | P1 | .... | .... | P4 | .... | .... | C2 | .... | .... | P4 |
| | R | .... | .... | .... | .... | .... | C2 | .... | .... | .... | .... | .... | .... |
| *Pontederia cordata* L..... | L | C1 | P1 | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Ranunculaceae | | | | | | | | | | | | | |
| *Aquilegia australis* Small..... | L | .... | .... | .... | .... | .... | .... | .... | .... | C1 | .... | .... | .... |
| **Clematis dioscoreifolia* Levl. and Vaniot..... | L | C2 | C1 | C5 | .... | .... | .... | .... | .... | C5 | C3 | .... | C5 |
| | St | .... | .... | C5 | .... | .... | .... | .... | .... | C5 | .... | .... | C5 |
| **Clematis* sp..... | L | .... | .... | .... | .... | .... | .... | .... | .... | C1 | .... | .... | .... |
| *Ranunculus septentrionalis* Poir.... | L | .... | .... | C2 | .... | .... | .... | .... | .... | C2 | .... | .... | P4 |
| *Viorna crispa* (L.) Small..... | L | C5 | C1 | C5 | .... | .... | C5 | C5 | C2 | C5 | C5 | C2 | C5 |
| Rubiaceae | | | | | | | | | | | | | |
| *Mitchella repens* L..... | L | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Rutaceae | | | | | | | | | | | | | |
| *Poncirus trifoliata* (L.) Raf..... | L | .... | .... | P5 | .... | .... | P5 | .... | .... | P5 | .... | .... | P5 |
| Sarraceniaceae | | | | | | | | | | | | | |
| *Sarracenia drummondii* Croom.... | L | .... | .... | .... | .... | P2 | .... | .... | .... | P2 | .... | .... | .... |
| *S. flava* L..... | L | .... | P2 | .... | .... | .... | .... | .... | .... | P2 | .... | .... | P1 |
| *S. mandiana* Hort. (*S. drummondii* Croom × *S. flava* L.)..... | L | .... | .... | .... | P1 | C1 | P2 | .... | .... | C2 | .... | .... | .... |
| Smilacaceae | | | | | | | | | | | | | |
| *Smilax glauca* Walt..... | L | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Tiliaceae | | | | | | | | | | | | | |
| *Tilia heterophylla* Vent..... | L | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Verbenaceae | | | | | | | | | | | | | |
| *Callicarpa americana* L..... | L | .... | .... | .... | C1 | C1 | .... | .... | .... | .... | C1 | .... | .... |
| | Fr | .... | .... | .... | .... | C1 | .... | .... | .... | .... | .... | .... | .... |
| *Lantana* sp..... | L, Fr | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| Vitaceae | | | | | | | | | | | | | |
| *Ampelopsis arborea* (L.) Rusby..... | L | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| *Muscadinia rotundifolia* (Michx.) Small..... | L | .... | .... | .... | .... | .... | .... | .... | .... | C2 | .... | .... | C2 |
| *Parthenocissus quinquefolia* (L.) Planch..... | L | .... | .... | .... | .... | .... | .... | .... | .... | C2 | .... | .... | .... |

presence or absence of an inhibitory factor. Millimeter readings of the inhibited zone gave only a very rough estimate of potency. The quantitative aspect is another problem to be studied in the development of an assay method for particularly promising plants uncovered in this survey.

Results reported in these studies are similar to those obtained by other workers. Carlson and Douglas (5) demonstrated that in testing for antimicrobial substances in chlorophyllose plants the type of solvent employed was important. Therefore, four different solvents were selected which would extract a wide variety of substances. In some instances the inhibiting material was present in all four of the extracts, but in others it was present in only one or two.

Osborn (17) reported that inhibitory properties might be present or absent, depending upon the plant part used. This is in agreement with the results reported here. In *Eichornia crassipes* (water hyacinth), for example, the leaf extracts in-

hibited the growth of *S. aureus* and *P. aeruginosa*, but the root and stem extracts were inactive against these two organisms. In accord with OSBORN, but contrary to the results of GOTTSHALL *et al.* (9), some seeds and fruits were found which possessed antimicrobial material; for example, extracts of the fruits of *Aleurites fordii* (tung oil tree) and *Albizzia julibrissin* (silk tree) and of seeds and fruits of *Hibiscus mutabilis* (cotton rose hibiscus) and *Crotalaria retzii* (Retz crotalaria) all caused inhibition of one or more of the test organisms.

BOAS and STEUDE (3) mentioned that a member of the Ranunculaceae contained a potent antimicrobial substance. OSBORN found that extracts of fifteen members of this family inhibited the growth of *S. aureus* and *E. coli*, and HAYES obtained her most potent extract from a species of the same family. In the present work two members of the Ranunculaceae—*Clematis dioscoreifolia* (yam-leaf clematis) and *Viorna crispa* (curly clematis)—were among the plants showing the most potent extracts.

As pointed out by SAUNDERS *et al.* (18), activity in a species does not necessarily indicate activity in all or any other species of the same genus. Extracts of *Sphagnum portoricense*, for example, were distinctly inhibitory to the test organisms, while those of *S. strictum* gave negative results. Although *Rhus toxicodendron* (poison oak) is irritating to the skin of human beings, extracts of this plant were not found to have antimicrobial powers. Yet extracts of *Rhus copallina* (dwarf sumac), a nonirritating plant closely related to *Rhus toxicodendron*, inhibited the growth of *C. albicans* to a marked extent.

The stability of the antimicrobial substances varied as was shown in a few repeat experiments not listed in table 1. *Sphagnum portoricense*, after being air-dried for 2 weeks, lost its activity against *S. aureus* and *P. aeruginosa*, although only a slight reduction of activity against *C. albicans* occurred. With a refrigerated extract of *Euphorbia pulcherrima* (common poinsettia) the marked inhibitory power was destroyed within 5 days when *C. albicans* was used as the test organism. A few experiments on the stability of the inhibiting substance in *Clematis dioscoreifolia* gave results considered worthy of special note. Although leaves of this plant when air-dried for 2 weeks lost all activity, extracts prepared from fresh leaves and kept in a refrigerator remained potent for at least 2 months.

OSBORN (17) noted that the antimicrobial substance was not always active against both test organisms employed. This was found to be true in the present experiments. More extracts were active against *C. albicans* than against either of the other two test organisms, although the total number of preparations inhibiting the growth of the gram-negative organism (*P. aeruginosa*) was almost the same as the total number inhibiting the gram-positive organism (*S. aureus*). Eight species were found, however, which were active against *S. aureus* and/or *P. aeruginosa* and not against *C. albicans*. Of these eight, two inhibited *S. aureus* alone—*Crotalaria retzii* (Retz crotalaria) and *Liriodendron tulipifera* (tulip tree); three inhibited *P. aeruginosa* alone—*Pontederia cordata* (pickerel weed), *Bigonia radicans* (trumpet vine), and *Dendropogon usneoides;* and three inhibited both *S. aureus* and *P. aeruginosa*—*Callicarpa americana* (American beauty berry), *Ipomoea purpurea* (common morning-glory), and *Nama quadrivalve* (Hydrolea).

Since *Clematis dioscoreifolia* has shown a very high degree of inhibiting activity against all three test organisms, it has been selected for further studies now being carried out by the authors.[1]

## Summary

1. The results of a survey for the occurrence of antimicrobial substances in chlorophyllose plants growing in Florida have been reported.

2. The tests were made for inhibitory activity against *Staphylococcus aureus* (P209), *Pseudomonas aeruginosa*, and *Candida albicans*.

3. Extracts of 102 species involving 126 plant parts and comprising over 1500 solutions were tested. Fifty-eight of the 102 species showed inhibitory power against one or more of the test organisms.

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# EFFECTS OF PHOSPHORUS DEFICIENCY ON GROWTH AND METABOLISM OF BLACK MUSTARD

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 631

SCOTT V. EATON

## Introduction

This is the third and final paper of a series pertaining to the effects of phosphorus deficiency on the growth and metabolism of plants. The first study (3) indicated that, although a shortage of phosphorus caused chlorosis of the leaves and a marked stunting of the entire plant, it did not seriously affect the metabolism of sunflower stems. Carbohydrates accumulated in the minus-phosphorus stems, especially in the early stages, but none of the soluble nitrogen fractions was high. The accumulation of carbohydrates seemed to be due to a greater leaf-stem, fresh-weight ratio of the phosphorus-deficient plants in comparison with the ones on complete nutrient rather than to any disturbance in their metabolism.

On the other hand, phosphorus deficiency very seriously affected the metabolism of soybean stems (4). They were high in sugars—especially at the early stages—starch, total soluble nitrogen, ammonia, and amides. Interruption of protein synthesis at the amide stage and proteolysis seemed to account for the accumulation of total soluble nitrogen and amides, and this disturbance was thought to explain the high carbohydrate content of the phosphorus-deficient stems.

It will be shown below that the effects of phosphorus deficiency on black mustard are similar to those obtained for sunflower and in contrast to the effects on soybean.

Since this paper completes the project, in the discussion the effects of a shortage of phosphorus on plants in general are reviewed with especial emphasis on the three plants of these studies.

## Material and methods

Black-mustard plants, *Brassica nigra* (L.) Koch, were grown in pure quartz sand by methods previously described (3). On cloudy days, of which there were a considerable number, natural daylight was supplemented from 8:00 A.M. until 5:00 P.M. by 200-watt incandescent Mazda bulbs placed 2 feet above the plants. Because of the drastic effects of phosphorus deficiency on growth, the plants later to be grown with a minus-phosphorus solution were supplied with phosphorus for 19 days after planting the seed. This was necessary in order to obtain enough tissue for analysis.

The seed was planted on March 13, 1951, and the plants were harvested April 19 and May 12 (37 and 60 days after planting). The number of plus- and minus-phosphorus plants of the first harvest was 229 and 109, respectively, and of the second, 114 and 58.

Essentially the same methods as previously described (3) were used in sampling, extraction, and chemical analysis. As in the soybean study (4), sodium hydroxide was used in obtaining alkalinity in the determination of ammonia, amides, and nitrates.

## Results

APPEARANCE AND SIZE.—Nine days after beginning the no-phosphorus treatment the leaves of many minus-phosphorus plants were greener than those of plus-phosphorus plants. No difference in stems, but this pigment was not evident in plus-phosphorus plants.

FIG. 1.—Black-mustard plants at time of first harvest, 37 days after planting seed. *Left*, minus-phosphorus; *right*, plus-phosphorus.

The phosphorus-deficient plants were smaller than the ones on complete nutrient and had smaller leaves and thinner stems. At the second harvest the size differences were quite pronounced (table 1, and figs. 1, 2). No difference in hardness of stems of the two groups was apparent.

In preliminary work plants were grown from planting time with a minus-phosphorus solution. They grew very little. After 42 days the tops of minus-phosphorus plants averaged only 0.102 gm.

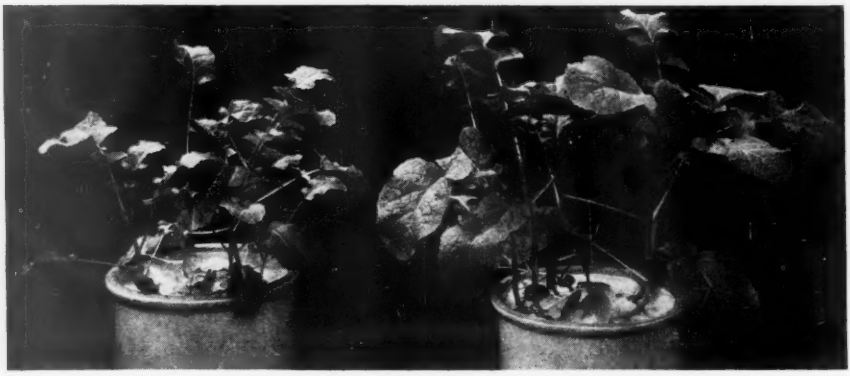

FIG. 2.—Black-mustard plants at time of second harvest, 60 days after planting seed. *Left*, minus-phosphorus; *right*, plus-phosphorus.

size of plants of the two groups was yet apparent. Later the difference in greenness became general and more pronounced. Though chlorosis was not a prominent effect of phosphorus deficiency, some lower leaves of both phosphorus-deficient and complete-nutrient plants became chlorotic. Although more prominent in the former plants than in the latter, the difference was not pronounced. A few minus-phosphorus plants developed a small amount of anthocyanin in the hypocotyls and lower

per plant compared with 17.86 gm. for tops of plus-phosphorus individuals. Yet chlorosis was little if any more prominent in the former than in the latter. Only a few phosphorus-starved plants developed anthocyanin, the stems and leaves of these becoming quite red. No plants on complete nutrient developed the pigment.

FRESH WEIGHT OF STEMS, PERCENTAGE WATER, AND PERCENTAGE DRY WEIGHT.—These are given in table 1. Since phosphorus deficiency had a similar effect on

fresh weights of leaves and roots as on stem weights, those data are not given.

Minus-phosphorus stems were slightly lower in moisture (higher in percentage dry weight) than those with phosphorus. The former weighed less than the latter, the difference becoming quite great by the second harvest. Phosphorus deficiency affected weight of tops more than that of roots, causing a lower top-root, fresh-weight ratio for the phosphorus-starved than for the plants on complete nutrient. These ratios for the minus-phosphorus and plus-phosphorus plants were 4.868 and 6.175, respectively, at the first harvest and 5.574 and 13.188 at the second. Shortage of phosphorus affected weight of stems slightly more than that of leaves, so that leaf-stem, fresh-weight ratios of phosphorus-starved plants were larger than those of plants on complete nutrient. These ratios for the first and second harvests were 7.010 and 3.228, respectively, for minus-phosphorus plants and 6.631 and 2.947 for plus-phosphorus plants. The ratios show that the leaves weighed much more than the stems in each group.

TABLE 1

FRESH WEIGHT OF WHOLE STEMS (GM/100 PLANTS), PERCENTAGE WATER, PERCENTAGE DRY WEIGHT

| Solution | Fresh weight | Percentage water | Percentage dry weight |
|---|---|---|---|
| | First harvest | | |
| Minus P..... | 38 | 92.3 | 7.7 |
| Plus P..... | 63 | 93.3 | 6.7 |
| | Second harvest | | |
| Minus P..... | 222 | 93.0 | 7.0 |
| Plus P..... | 622 | 93.8 | 6.2 |

CHEMICAL COMPOSITION.—Minus-phosphorus stems were lower in soluble solids, total nitrogen, soluble nitrogen, and insoluble nitrogen than stems on complete nutrient (table 2), although differences in the nitrogen fractions were small. There was about the same percentage of ammonia, somewhat more amides, and less nitrate in the former than in the latter stems of the second harvest (table 3).

There was not much difference in the percentages of total sugars, reducing sugars, and starch in the stems of the two groups at the first harvest (table 4). There was only a trace of sucrose in the plus-phosphorus and only 0.12% in the

TABLE 2

SOLUBLE SOLIDS, TOTAL N, SOLUBLE N, INSOLUBLE N
(Percentages on dry-weight basis)

| Solution | 70% alcohol-soluble solids | Total N | 70% alcohol-soluble N | 70% alcohol-insoluble N |
|---|---|---|---|---|
| | First harvest | | | |
| Minus P.... | 32.7 | 4.47 | 3.04 | 1.43 |
| Plus P..... | 34.5 | 4.92 | 3.40 | 1.52 |
| | Second harvest | | | |
| Minus P.... | 32.3 | 4.01 | 2.96 | 1.05 |
| Plus P..... | 36.0 | 4.69 | 3.51 | 1.18 |

TABLE 3

AMMONIA-, AMIDE-, AND NITRATE-NITROGEN
(Percentages on dry-weight basis, second harvest)

| Solution | Ammonia | Amide | Nitrate |
|---|---|---|---|
| Minus P..... | 0.061 | 0.127 | 2.652 |
| Plus P..... | 0.067 | 0.111 | 3.128 |

minus-phosphorus stems. At the second harvest all the sugars were higher in the minus-phosphorus stems, but the starch content of the two sets was about the same.

TABLE 4

CARBOHYDRATE FRACTIONS
(Percentages on dry-weight basis)

| Solution | Total sugars | Reducing sugars | Sucrose | Starch |
|---|---|---|---|---|
| | First harvest | | | |
| Minus P.... | 0.86 | 0.74 | 0.12 | 0.68 |
| Plus P..... | 0.72 | 0.71 | 0.01 | 0.70 |
| | Second harvest | | | |
| Minus P.... | 2.38 | 1.61 | 0.77 | 0.71 |
| Plus P..... | 1.38 | 1.12 | 0.26 | 0.65 |

TABLE 5

PHOSPHORUS FRACTIONS
(Percentages on dry-weight basis)

| Solution | Total P | 70% alcohol-soluble P | 70% alcohol-insoluble P |
|---|---|---|---|
| | First harvest | | |
| Minus P..... | 0.183 | 0.075 | 0.108 |
| Plus P..... | 0.465 | 0.147 | 0.318 |
| | Second harvest | | |
| Minus P..... | 0.104 | 0.044 | 0.060 |
| Plus P..... | 0.484 | 0.119 | 0.365 |

All the phosphorus fractions were much lower in the phosphorus-starved than in the stems on complete nutrient (table 5). More of the total phosphorus was present in soluble form in the former than in the latter stems, the difference being greater at the second harvest. The ratios of soluble phosphorus to total phosphorus for the minus-phosphorus and plus-phosphorus stems were 40.983 and 31.613, respectively, for the first harvest, and 42.307 and 24.587 for the second.

## Discussion

In this final paper it seems pertinent to review the effects of phosphorus deficiency on plants in general with special emphasis on the three plants of this project: soybean, sunflower, and black mustard.

### APPEARANCE AND SIZE

The main symptoms of phosphorus deficiency in plants are the deeper-green color of the leaves and a general stunting in comparison with plants grown with complete nutrient. All three species studied showed these symptoms. Increased greenness was evident before there was much if any limitation in size. Other investigators have obtained similar results (**10, 14**). MCMURTREY (**14**) studied the effects on field-grown tobacco plants of deficiencies of nine essential chemical elements. In the case of phosphorus deficiency he also reported the retardation of growth and the dark-green color of the leaves in comparison with plants on complete nutrient. It was emphasized that the effects of phosphorus deficiency on growth were less characteristic than those resulting from the shortage of any other element.

Phosphorus deficiency retarded growth of the tops of sunflower, soybean, and black-mustard plants more than that of the roots, so that top-root, fresh-weight ratios of minus-phosphorus were lower than those of plus-phorphorus plants. This agrees with much of the literature (**6, 16**). However, TURNER (**17**) found that increasing the amounts of phos-

phorus in the nutrient solution caused a decrease in the top-root ratios of barley, wheat, and cotton. In discussing TURNER's conflicting results, GOEDEWAGEN (6) and SOMMER (16) emphasized the high concentration of phosphate in his nutrient solutions.

Stems of phosphorus-starved plants are usually hard and woody, and this situation is usually correlated with a high carbohydrate and a low water content. ECKERSON (5) found that the cell-wall thickness in collenchyma, xylem parenchyma, and pith of minus-phosphorus tomato stems was greater than that of plus-phosphorus ones and that the amount of collenchyma was also greater. The former stems had a higher carbohydrate percentage than the latter. In the present series of studies no anatomical work was done, but the phosphorus-starved sunflower and soybean stems were harder than those on complete nutrient and on analysis revealed a lower water and a higher carbohydrate content. On the other hand, phosphorus deficiency did not seem to result in greater hardness of black-mustard stems. There was little difference in water content of the stems of the two groups (table 1). Although at the second harvest minus-phosphorus stems were considerably higher in sugars than those with phosphorus, the starch content was about the same in stems of the two sets (table 2). The carbohydrate content of phosphorus-starved sunflower and soybean stems relative to those on complete nutrient was much higher than in black-mustard stems.

In the literature chlorosis is not reported as a prominent feature of phosphorus deficiency. MCMURTREY (14) did not observe chlorosis in tobacco grown in phosphorus-deficient soils, and even in plants grown in no-phosphorus nutrient solutions (13) only a few of the lower leaves became yellow. HAAS (9) noted the chlorosis and burning of citrus leaves resulting from phosphorus shortage. In the three species of this project chlorosis of the lower leaves following the early dark-green color was prominent in sunflower, much less so in soybean, and scarcely evident in black mustard.

Anthocyanin development is often a feature of phosphorus deficiency (5, 11). Minus-phosphorus sunflower and soybean plants did not develop the pigment, and it was observed in only a few black-mustard plants. Anthocyanin formation may be regarded as a secondary symptom of phosphorus deficiency resulting from the high carbohydrate content of the plants and from the presence of the proper genotype for development of the pigment. Apparently the experimental sunflower and soybean plants cannot form anthocyanins; black mustard does, but the carbohydrate content was probably not high enough for marked anthocyanin formation.

## CHEMICAL COMPOSITION

CARBOHYDRATE FRACTIONS.—A number of investigators (5, 7, 12) have reported that accumulation of carbohydrates seems to be a uniform effect of phosphorus deficiency on plants. All three species of this project showed this effect. The accumulation varied with kind of plant, kind of carbohydrate, and the severity of the deficiency. In both soybean and sunflower stems the accumulation of reducing sugars and sucrose was much greater early in the deficiency than later. This was also true of starch in sunflower stems, but this carbohydrate continued to accumulate in soybean stems throughout the entire experiment. Phosphorus deficiency did not cause so great an accumulation of

carbohydrates in black-mustard stems as in those of sunflower and soybean. However, neither were such severe deficiency symptoms obtained in the first-named plant as in the other two. Reducing sugars and sucrose were considerably higher in minus-phosphorus than in plus-phosphorus black-mustard stems, but the starch content was about the same in stems from the two groups.

Certain investigators have found that starch breaks down at severe stages of phosphorus deficiency. ECKERSON (5) noted an accumulation of starch and sugars in phosphorus-starved tomato plants. When the effects of a shortage of phosphorus became quite drastic, starch along with other phosphorus-containing compounds began to break down. Sugars increased rapidly. KRAYBILL (10) found that at late stages minus-phosphorus tomato plants contained less starch than the controls. There was not much evidence of a breakdown of starch in the three plants of these studies. Starch continued to increase in minus-phosphorus soybean stems throughout the experiment. The starch content of phosphorus-starved black-mustard stems was about the same as that of the controls at each harvest. Although the percentage of starch in minus-phosphorus sunflower stems was less at the third harvest than at the second, it was as high as the controls when figured on the dry-weight basis, and considerably higher when calculated on the wet-weight basis. The starch content of control stems was also less at the third harvest than at the second. The decrease in the percentage of starch in stems of the two groups may have been more apparent than real and due perhaps to the increase in nonstarch constituents.

There are two main explanations in the literature for the accumulation of carbohydrates in phosphorus-starved plants: (*a*) interference with protein synthesis at the nitrate-reduction stage, resulting in the increase of both carbohydrates and nitrates (5), and (*b*) interference with protein synthesis at the amide stage with the resultant accumulation of carbohydrates, amides, and amino acids (15). The first explanation does not obtain in the plants of this project. Nitrates were higher in the plus-phosphorus than in the minus-phosphorus stems of each of the three species. There was no apparent interference with nitrate reduction. The second explanation seemed to account for the accumulation of carbohydrates in soybean stems, for amides were high in the phosphorus-starved stems of this plant. Amino acids were not determined, but they also probably accumulated, since concentration of amino acids and amides usually run more or less parallel. Neither explanation accounted for the accumulation of carbohydrates in minus-phosphorus sunflower and black-mustard stems, although in the latter there seemed to be a slight interference with protein synthesis at the amide stage. At the final harvest there were somewhat more amides in the phosphorus-starved stems than in the controls. There was no measurable amount of amides in sunflower stems at the second harvest, when deficiency symptoms were very marked. Nitrates did not accumulate in the stems of either plant. Hence, in these plants protein synthesis was disturbed apparently at neither the nitrate-reduction nor the amide stage except as mentioned above for the black mustard.

The leaf-stem fresh-weight ratio was higher in the minus-phosphorus than in the plus-phosphorus stems of black mustard at each harvest. This was true, too, of the sunflower plants of the first harvest. GREGORY and RICHARDS (8) found that phosynthesis in young phosphorus-starved barley leaves was slightly

more rapid than in those on complete nutrient. This may also be true of the plants of this project. The greater weight of leaves in proportion to stems and the higher rate of photosynthesis—rather than any disturbance in the metabolism—may partly account for the high carbohydrate content of the phosphorus-starved black-mustard stems throughout the experiment, and of the sunflower stems, especially in the early part of the deficiency. There was less difference in the carbohydrate contents of the two sets of sunflower stems late in the deficiency, and at certain levels it was higher for those on plus-phosphorus. In line with this situation the leaf-stem fresh-weight ratio of the phosphorus-starved sunflower plants was less than that of those on complete nutrient at the third harvest, owing partly to death of some leaves. Also, as the deficiency became more severe, the rate of photosynthesis probably decreased, as GREGORY and RICHARDS (8) found in barley leaves. The reduced weight of leaves in proportion to the stems and the lower rate of photosynthesis may partly explain why late in the deficiency there was less difference in the carbohydrate content of the minus-phosphorus and plus-phosphorus stems.

NITROGEN FRACTIONS.—A number of investigators have found that phosphorus deficiency caused the accumulation in plants of water-soluble forms of nitrogen, including nitrates, ammonia, amino acids, and amides (5, 10, 12, 15). In the three species of this project nitrogen metabolism was seriously disturbed by phosphorus deficiency only in the soybean. Phosphorus-deficient stems of this plant were high in total soluble nitrogen, ammonia, and amides in comparison with those on complete nutrient. Amino acids were not determined but presumably also accumulated, since concentrations of amino acids and amides in plants are usually rather similar. There was a slight accumulation of amides in the minus-phosphorus black-mustard stems of the second harvest, but total soluble nitrogen and ammonia were higher in the stems on complete nutrient. Nitrates did not accumulate in stems of any of the phosphorus-starved plants of this series of studies except at the lower level of the soybean stems, but even in this plant nitrates calculated for whole stems were much higher in the plus-phosphorus stems.

ECKERSON (5) thought the accumulation of nitrates in phosphorus-starved tomato plants was a result of the interference with the reduction of nitrates owing to the loss of reducase activity. On the other hand, BREON and GILMAN (1) presented data indicating that the high nitrate content of tomato plants showing definite but moderate phosphorus-deficiency symptoms was due to more rapid absorption rather than to nonutilization. As stated above, nitrates did not accumulate in the minus-phosphorus stems, in comparison with plus-phosphorus stems, of any plants of this project.

Two possible explanations of the accumulation of amino acids and amides in phosphorus-starved plants were given by ECKERSON (5). They may have been formed early when there was still some reducase activity but when certain conditions necessary for their synthesis into proteins were lacking; or they may have resulted from proteolysis late in the deficiency. RICHARDS and TEMPLEMAN (15) explained the high content of barley leaves in these compounds by the need of phosphorus for their synthesis into proteins. This corresponds essentially to the first explanation of ECKERSON, if we assume that phosphorus is the lacking material necessary for protein synthesis.

As indicated above, only in the minus-

phosphorus soybean stems did amides (and presumably amino acids) accumulate to any great extent. Both of the explanations of ECKERSON seem necessary to account for their accumulation. Owing to the shortage of phosphorus, the synthesis of nucleo-and cytoplasmic proteins would be interrupted at the amide stage so that both amides and amino acids would accumulate. But proteolysis seemed also to be occurring. Evidence for this was based mainly on the gradients of percentage content. The amide gradient was positive downward (decreasing concentration from top to bottom) in the plus-phosphorus stems, but for minus-phosphorus stems the highest concentration was at the middle level. At the lower level of the latter stems the concentration was higher than at the upper. This gradient is regarded as evidence that proteolysis was occurring in the lower and middle parts of the phosphorus-starved stems and that the resulting material was beirg translocated to the tops and used in growth.

PHOSPHORUS FRACTIONS.—In all plants of this project the total soluble phosphorus made up a considerably larger percentage of the total phosphorus of minus-phosphorus than of plus-phosphorus stems. This may be regarded as evidence for the breakdown of phosphorus-containing compounds and the possible re-utilization of the resulting phosphorus. The soluble phosphorus was not fractionated. DETURK (2) fractionated the phosphorus compounds of the corn plant, determining their concentration during the development of the plant. He concluded that the phosphates and acid-soluble organic phosphorus compounds were the main translocatable forms. ECKERSON (5) found that about 5 weeks after the beginning of a no-phosphorus treatment, when phosphorus deficiency was very severe, phosphorus-containing compounds of the tomato plant began to break down in the order: starch, phosphatides, proteins. Soon the protoplasm became disrupted and the cells disorganized. Even if the proportion of disorganized cells was small, the addition of phosphate was not beneficial. The plant did not recover.

No attempt was made in these studies to determine how long the plants would remain alive when grown with a no-phosphorus solution. Black-mustard plants were grown a maximum of 41 days without phosphorus, soybean 55 days, and sunflower 64 days. The sunflower plants were then suffering from extreme phosphorus deficiency, only the leaves quite near the top being normal, but all the plants developed small flower buds. The sunflower can apparently be grown to maturity with a phosphorus-deficient nutrient solution. Perhaps this plant can more readily re-utilize the phosphorus of complex organic compounds than can corn or tomato.

## Summary

1. Sand cultures were used in a study of the effects of phosphorus deficiency on black-mustard plants (*Brassica nigra* [L.] Koch). The first symptom observed was the deeper-green color of the leaves. Neither chlorosis nor anthocyanin development was prominent, although some of the lower leaves became chlorotic, and a few plants developed a small amount of anthocyanin in the hypocotyls and stems. The deficient plants were short with small leaves and thin stems. No difference in hardness of the stems of the two groups was apparent. Top growth was limited more than root growth, so that the top-root fresh-weight ratio of minus-phosphorus was lower than that of plus-phosphorus plants.

2. Phosphorus-deficient stems were slightly lower in moisture than those on complete nutrient. The former stems were high in total sugars, reducing sugars, and sucrose but contained about the same amount of starch as the latter stems. The accumulation of sugars is interpreted as being a consequence of the greater leaf-stem fresh-weight ratio of the minus-phosphorus as compared with the plus-phosphorus plants. A small part of the accumulation seemed to be due to a slight disturbance of protein synthesis at the amide stage.

3. Phosphorus deficiency did not seriously disturb the nitrogen metabolism of black-mustard stems. There was a slight accumulation of amides in the minus-phosphorus stems; total nitrogen, soluble nitrogen, and nitrates were higher in the plus-phosphorus ones. The concentration of ammonia was about the same in stems of the two sets.

4. Total phosphorus, soluble phosphorus, and insoluble phosphorus were much higher in plus-phosphorus than in phosphorus-deficient stems. The soluble phosphorus made up a much larger percentage of the total phosphorus in the latter than in the former stems.

5. The effects of phosphorus deficiency on plants in general are discussed with especial emphasis on a comparison of the three species of this project.

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# EVIDENCE ON THE AUXIN-ETHYLENE BALANCE HYPOTHESIS OF FOLIAR ABSCISSION[1]

WAYNE C. HALL

## Introduction

Leaf-fall occurs naturally in a large variety of plants, yet the basic chemical and physiological factors underlying this phenomenon are still relatively obscure. Reference to the physiological literature indicates clearly the paucity of our present knowledge and the lack of fundamental research in the field. Recent papers by GAWADI and AVERY (7) and by LIVINGSTON (18) have pointed out that, although abscission has been studied since the middle of the eighteenth century, the larger part of the published work has been confined to anatomical observations, whereas the physiological experiments undertaken have been mostly concerned with abscission of immature reproductive organs and preventive measures to control fruit-drop.

During the last decade the mechanization of cotton-picking as well as the machine harvesting of other important crops has created a practical necessity for better understanding of the physiological factors leading to success or failure in the artificial defoliation of plants. Some of the reported factors instrumental in accelerating abscission—such as a water deficit, extremes of temperature, reduced light intensity, ethylene and other chemicals, destruction of all or part of the leaf blade by physical factors, insects, and fungi, etc.—have been adequately reviewed recently by GAWADI and AVERY (7) and LEE and CAROLUS (17); yet the actual mechanism whereby any of the above factors or conditions induces leaf-fall is still largely unknown.

LIVINGSTON (18) tested various materials by means of the citrus-explant technique; only ethylene accelerated abscission. Employing the same technique, BROWN and ADDICOTT (5) confirmed this observation for Black Valentine bean explants and noted that ethylene induced rapid cellular disintegration in the abscission zone. SHOJI *et al.* (25) have established that an auxin gradient exists across the abscission zone of the Black Valentine bean while the leaf is active but that this gradient is lost immediately preceding abscission.

During the last 4 years studies of the biological origin of ethylene, of its effects upon the growth and development of plants, and of other aspects of this active emanation (10, 11) have been undertaken by the author. During the course of these investigations the effect of ethylene on defoliation has been observed. Recently, in their study of leaf abscission, GAWADI and AVERY (7) proposed a theory to explain natural abscission in terms of physiological circumstances in the leaf prior to leaf-fall. This hormone-ethylene balance hypothesis reflects almost exactly the author's views on the subject and has been proposed almost in identical form to students for several years, as a possible theoretical explanation of the process.

The present study reports some of the

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more pertinent experimental evidence collected in support of the auxin-ethylene balance hypothesis.

## Material and methods

THE QUICK PETIOLE TEST.—SAMPSON (**24**) noted that, if the blade of a *Coleus* leaf is removed, abscission of the petiole follows in 1–2 days. LA RUE (**15, 16**) showed that, if a small smear of lanolin containing indoleacetic acid (IAA) is placed on the end of a debladed *Coleus* petiole, abscission of that petiole is greatly retarded as compared with petiole stumps treated only with pure lanolin. This suggested that the blade normally supplies a growth hormone, possibly IAA, which prevents abscission of the petiole.

Experimentation revealed that the debladed petiole test was easily adaptable as a quick screening technique to assay the effectiveness of IAA and other compounds in the abscission process. The compounds to be tested either singly or in combination were weighed out to the desired concentration and taken up in a minimum of a suitable organic solvent (ether, ethanol, etc.). This was then stirred into 10 gm. of melted lanolin on a hot plate and heated slowly to volatilize the solvent. The paste was also stirred occasionally while hardening and left uncovered overnight prior to using. Volatile liquids, such as ethylene chlorohydrin, were stirred, without heating, directly into melted lanolin as it started to harden. The lanolin paste containing the test reagent was then applied to the debladed petiole stump as a small smear with a glass applicator. Following the application, daily counts were made of the number of petioles which abscised unaided as well as those that fell when gentle pressure was applied. The numbers shed, however, are reported only at the end of 2 or 3 and 7 days (tables 1, 2).

In tests with *Coleus* mature healthy

TABLE 1

EFFECTS OF VARIOUS CHEMICALS IN LANOLIN ON ABSCISSION OF DEBLADED PETIOLES OF MATURE COLEUS PLANTS

| Variable | No. petioles treated | No. abscised in 2 days | No. abscised in 7 days | % total abscission | Remarks |
|---|---|---|---|---|---|
| Control | 20 | 6 | 15 | 75 | Leaves debladed |
| Lanolin only | 20 | 7 | 19 | 95 | Abscission effect from lanolin; late abscission |
| 1% IAA* | 20 | 0 | 0 | 0 | Petioles firmly attached |
| 1% Ethylene chlorohydrin | 20 | 18 | 20 | 100 | Complete abscission in 3 days |
| 1% Ethylene chlorohydrin—1% IAA | 28 | 0 | 2 | 7 | Eventually complete abscission by 14 days |
| 3% Endothal† | 20 | 12 | 20 | 100 | Complete abscission by 5 days |
| 1% Endothal—1% IAA | 28 | 0 | 3 | 10 | Petioles firmly attached |
| 3% Sodium chlorate-pentaborate | 20 | 5 | 13 | 65 | Killed tissues, toxic concentration |
| 1% Sodium chlorate-pentaborate —1% IAA | 26 | 0 | 0 | 0 | Petioles firmly attached |
| 3% Trans-cinnamic acid | 26 | 7 | 22 | 84 | Toxic concentration; abscission before controls, at 5 days |
| 1% Trans-cinnamic acid—1% IAA | 20 | 0 | 3 | 15 | Petioles firmly attached |

* Indoleacetic acid.

† Disodium 3,6-endoxohexahydrophthalate.

plants were employed, and only the larger leaves on one side of the primary shoot were debladed and treated. Extreme basal leaves as well as the two leaves originating from the apical nodes were not used in the test. By leaving the leaves intact on one side of the plant, impairment of normal metabolism was assumed to be reduced.

In experiments with cotton, plants at the early square (flower primordia) stage were utilized in the petiole tests. Normal intact cotton leaves at this stage of development ordinarily are extremely difficult to defoliate with common commercial defoliants. Every other leaf, excluding the cotyledons and the two uppermost leaves, was debladed and treated. By leaving every other leaf blade intact, it was thought that normal metabolism would not be drastically interfered with and that the plants would more nearly approach the condition of intact plants.

LIVINGSTON (**18**) has recently reported an *in vitro* agar explant test using excised citrus-leaf tissue, which has much in its favor, and which apparently gives results comparable with those obtained in the field. It is believed, however, that the intact petiole test is as easy to use and yet more nearly simulates conditions under which abscission naturally takes place.

EXPERIMENTS WITH ETHYLENE GAS.—The effects of ethylene gas upon defoliation, when used alone and in combination with the sodium salt of naphthaleneacetic acid (sodium NA), were determined on mature cotton plants, Stoneville 2B variety. Plants were selected, cut off at the ground level, and inclosed under large bell jars with the gas. The ethylene was generated by the dehydration of ethyl alcohol according to the well-known method and was employed at 100 and 200 p.p.m. in the experiments summarized in table 3. Some of the plants were sprayed twice, 24 hours apart, with 1000 p.p.m. sodium NA until the leaves were wet and then left in the greenhouse an additional 24 hours prior to using in the bell jars. With other plants the ends of the cut stems were placed in aqueous solutions of sodium NA at either 1500 or 500 p.p.m. concentration for 24 hours before the tops were inclosed with ethylene. The amount of defoliation was determined in all cases after 48 hours of exposure to the ethylene gas.

DETERMINATION OF ETHYLENE EVOLUTION.—The method employed for estimation of ethylene evolution is a potassium permanganate reduction technique adapted after the procedure of NELSON (**20**) and reported in detail elsewhere (**11**). The amount of ethylene produced by 100 gm. of healthy mature cotton leaves was determined by the above method as follows: Greenhouse-grown plants were sprayed to runoff with aqueous solutions of Sharples' Endothal (disodium 3,6-endoxohexahydrophthalate plus ammonium sulfate), Chipman's Shed-A-Leaf (sodium chlorate-pentaborate), or trans-cinnamic acid by means of a hand-atomizer sprayer. A commercial wetting agent, Nonic 218, was added to all solutions. The first two compounds are commercial defoliants while trans-cinnamic acid has been reported to be an anti-auxin (**27**). When leaves of the sprayed plants were thoroughly dry, 100-gm. samples from each treatment were removed, and the petioles were placed in beakers of water. The samples from each treatment were then placed separately in the evacuation chamber, and the amount of ethylene and carbon dioxide produced was measured during a 72-hour period. Barium hydroxide scrubbing towers to absorb carbon dioxide were placed between the respiring leaves and the potassium permanganate receiving flask. The

absorbed carbon dioxide was determined by HCl titration at the end of the run; it served as an approximate index to the rate of respiration and is reported only as such in the data. A comparable lot of untreated leaves was similarly tested; their ethylene and carbon dioxide production represents the amount emanated under essentially normal conditions and serves as a basis of comparison for the treated leaves.

The pea seedlings used in the "triple response test" were germinated according to the method of PRATT and BIALE (22) and were placed above the test solutions contained in the bottom of large sealed desiccators. Observations were made at the end of 72 hours. The enzyme source employed in these tests was obtained by aqueous-dilute acetone extraction of *Penicillium digitatum* cultures as previously described (11).

## Results

PETIOLE TESTS WITH *Coleus*.—The results of two separate experiments with mature *Coleus* plants have been combined and summarized (table 1). Debladding of the leaves, without any other treatment, resulted in 75% abscission of the petioles within a week, whereas plants with normal intact leaves shed less than 1% of their leaves during the same period. Thus the leaf blade does exert some inhibitory effect on abscission; this agrees with results reported by LIVINGSTON (18), who found a high correlation between percentages of inhibition of abscission and amount of leaf blade present. Lanolin by itself apparently has some accelerating effect on abscission as the *Coleus* petioles receiving this treatment had a higher rate of abscission than the controls. This was also found with young cotton petioles (table 2), although the effects were less pronounced. REDEMANN *et al.* (23) have reported that lanolin is not a completely inert solvent as often assumed and may contain oxidizing agents capable of inactivating or destroying IAA. Partial destruction of the natural IAA of the petioles by lanolin may therefore account for some of its effect on abscission.

Applications to the petiole stumps of lanolin containing 1% IAA completely prevented abscission. This indicates, as others have previously suggested, that this type of hormone is the factor supplied by the blade that normally prevents abscission. Treatment of *Coleus* petioles with either ethylene chlorohydrin or Endothal brought about complete abscission within 3 or 5 days, respectively, following application (table 1); at the end of 2 days there was marked acceleration of the defoliation process compared with other treatments. Sodium chlorate-pentaborate, under the experimental conditions employed, apparently was not so potent a defoliant, although the data for cotton (table 2) indicate that this substance does accelerate abscission. The concentration of sodium chlorate-pentaborate used for *Coleus* was much too high and resulted in death of some of the petioles.

Petioles treated with trans-cinnamic acid had a higher abscission rate than the controls. However, when the commercial defoliants, ethylene chlorohydrin, or trans-cinnamic acid were combined with an equal concentration of IAA, petiole-drop was greatly retarded in all cases (table 1).

PETIOLE TESTS WITH COTTON.—More extensive experiments were conducted with young preflowering cotton, and the results from three separate applications made during the months of October, November, and December are summarized in table 2. The controls, which were de-

bladed only, had a much lower abscission rate than *Coleus* over the same period of time. This may be due to the greater petiole length of cotton, a higher natural IAA content, or other factors yet to be explained. As was true for *Coleus*, the IAA treatment completely inhibited abscission. Increasing the concentrations of Endothal, sodium chlorate-pentaborate, trans-cinnamic acid, and ethylene chlorohydrin had little or no stimulating effect on abscission rates. In the case of ethylene chlorohydrin, apparently even 0.5% concentration is adequate, while a 3% concentration becomes toxic. To check more closely the interaction of IAA and the other compounds, a wider range of concentrations was tested than was used

TABLE 2

EFFECTS OF VARIOUS CHEMICALS IN LANOLIN ON ABSCISSION OF DEBLADED PETIOLES OF YOUNG PREFLOWERING COTTON PLANTS. APPLICATIONS MADE ON GREEN-HOUSE PLANTS DURING OCTOBER, NOVEMBER, AND DECEMBER

| Variable | No. petioles treated | No. abscised in 3 days | No. abscised in 7 days | % total abscission | Remarks |
|---|---|---|---|---|---|
| Control | 31 | 0 | 3 | 9 | Most of petioles firmly attached |
| Lanolin only | 20 | 1 | 4 | 20 | Most of petioles firmly attached |
| 1% IAA* | 20 | 0 | 0 | 0 | No sign of abscission |
| 1% Endothal† | 24 | 17 | 19 | 80 | All petioles abscised in 9 days |
| 3% Endothal | 24 | 13 | 20 | 83 | Number of petioles died but abscised |
| 3% Endothal—1% IAA | 12 | 5 | 9 | 75 | Petioles alive |
| 1% Endothal—1% IAA | 38 | 7 | 11 | 30 | Unshed petioles firmly attached |
| 1% Endothal—0.1% IAA | 32 | 15 | 16 | 50 | Most of abscission early; remainder firmly attached |
| 1% Sodium chlorate-pentaborate | 29 | 12 | 15 | 52 | Unshed petioles firmly attached |
| 3% Sodium chlorate-pentaborate | 24 | 5 | 14 | 58 | Unshed petioles alive and firmly attached |
| 3% Sodium chlorate-pentaborate —1% IAA | 12 | 3 | 5 | 42 | Unshed petioles alive and firmly attached |
| 1% Sodium chlorate-pentaborate —1% IAA | 36 | 3 | 11 | 30 | Unshed petioles alive and firmly attached |
| 1% Sodium chlorate-pentaborate —0.1% IAA | 31 | 10 | 14 | 45 | Unshed petioles alive and firmly attached |
| 3% Ethylene chlorohydrin | 18 | 10 | 16 | 90 | Bulk of abscission within 4–5 days; slightly toxic |
| 1% Ethylene chlorohydrin | 26 | 17 | 24 | 92 | Early abscission; stimulated new growth |
| 0.5% Ethylene chlorohydrin | 12 | 4 | 11 | 92 | Some stimulation of new growth |
| 1% Ethylene chlorohydrin—1% IAA | 36 | 9 | 27 | 75 | |
| 1% Ethylene chlorohydrin— 0.1% IAA | 25 | 12 | 21 | 84 | |
| 0.5% Ethylene chlorohydrin— 0.1% IAA | 12 | 0 | 4 | 33 | |
| 1% Trans-cinnamic acid | 12 | 3 | 7 | 58 | Kills tissue; then abscission |
| 0.5% Trans-cinnamic acid | 12 | 2 | 6 | 50 | Less toxic concentration |
| 1% Trans-cinnamic acid—1% IAA | 14 | 2 | 4 | 29 | Tissue died but petioles firm |
| 0.5% Trans-cinnamic acid— 0.1% IAA | 10 | 3 | 4 | 40 | Unshed petioles firmly attached |

* Indoleacetic acid.

† Disodium 3,6-endoxohexahydrophthalate.

for *Coleus*. A ratio of 3% Endothal to 1% IAA resulted in 75% abscission, whereas equal 1% concentrations of the two compounds reduced abscission to 30%, while a mixture of 1% Endothal and 0.1% IAA gave 50% abscission. The same general trend in abscission was shown by the comparable series of treatments when IAA was mixed with sodium chlorate-pentaborate, ethylene chlorohydrin, or trans-cinnamic acid. The highest percentage of abscission, when using equal concentrations of IAA and defoliant, occurred in the ethylene chlorohydrin treatment. This strongly suggests that ethylene, in itself, has a marked effect on abscission and that possibly the effect of the other compounds was to accelerate ethylene production in the petioles.

TESTS WITH ETHYLENE GAS.—Because of the possible objection in the use of ethylene chlorohydrin as the source of ethylene, ethylene gas was also used in the experiments outlined in table 3. It was found, as in the petiole tests with *Coleus* and cotton, that ethylene was as effective or even more so than ethylene chlorohydrin in inducing an early and high amount of defoliation (table 3, exp.

TABLE 3

EFFECTS OF ETHYLENE GAS AND SODIUM NA, SINGLY AND IN COMBINATION, UPON ABSCISSION OF MATURE COTTON LEAVES

| Experiment no. | Variable | No. leaves observed | No. abscised in 48 hr. | % total abscission | Remarks |
|---|---|---|---|---|---|
| 1*a* | Control | 52 | 0 | 0 | Ends of cut stems placed in water under bell jars with air |
| 1*b* | 100 p.p.m. ethylene gas | 91 | 88 | 96 | Ends of cut stems placed in water under bell jars with ethylene; chlorosis of leaves noted |
| 2*a* | Control | 51 | 1 | 2 | Ends of cut stems placed in water under bell jars with air |
| 2*b* | 1000 p.p.m. sodium NA—100 p.p.m. ethylene gas | 64 | 6 | 9 | Plants sprayed twice, 24 hr. apart, with sodium NA; then left in greenhouse 24 hr. before placing under bell jars with ethylene; stems in water |
| 3*a* | 100 p.p.m. ethylene gas | 48 | 46 | 96 | Ends of cut stems placed in water 24 hr. prior to plants being placed under bell jars with ethylene |
| 3*b* | 1500 p.p.m. sodium NA—100 p.p.m. ethylene gas | 46 | 0 | 0 | Ends of cut stems soaked in sodium NA 24 hr. prior to plants being placed under bell jars, still in sodium NA with ethylene |
| 3*c* | 1500 p.p.m. sodium NA—100 p.p.m. ethylene gas | 68 | 15 | 22 | Ends of cut stems presoaked 24 hr. in sodium NA, then placed in water under bell jars with ethylene |
| 3*d* | 1500 p.p.m. sodium NA—100 p.p.m. ethylene gas | 46 | 4 | 9 | 3*b* plants in sodium NA re-treated for 48 additional hr. under bell jars with 1000 p.p.m. ethylene |
| 4*a* | 500 p.p.m. sodium NA—100 p.p.m. ethylene gas | 32 | 0 | 0 | Ends of cut stems presoaked in sodium NA for 24 hr., then placed in sodium NA under bell jars with ethylene |
| 4*b* | 500 p.p.m. sodium NA—200 p.p.m. ethylene gas | 30 | 10 | 33 | Ends of cut stems presoaked in sodium NA 24 hr. before placing in water under bell jars with ethylene |

1*a*, 1*b*). The rapidity of action of ethylene in inducing abscission has been reported by others (**5**, **18**). Other noteworthy effects observed in the experiments when a high rate of abscission occurred were: reddening of the petioles in the early stages of exposure, followed by chlorosis and total loss of color; extreme proliferation of soft white tissue at the base of stems and up the stems to leaves beginning about 24 hours after exposure to ethylene; early abscission of squares and flowers; proliferation of tissue at nodes and growing points; and buds forced at nodes.

balance between auxin (sodium NA) and ethylene is the deciding factor in the percentage of abscission effected.

ETHYLENE PRODUCTION BY COTTON LEAVES.—Table 4 summarizes a representative run showing the amount of ethylene produced by 100 gm. of untreated mature cotton leaves and by cotton leaves sprayed with Endothal, trans-cinnamic acid, or sodium chlorate-pentaborate. Determinations for the individual treatments shown were measured from three to six times each, and, although considerable deviation existed in the values obtained among separate runs,

TABLE 4

ETHYLENE PRODUCTION BY 100 GM. OF RESPIRING MATURE COTTON LEAVES DURING 72 HOURS FOLLOWING SPRAY APPLICATION

| Variable | Ml. of ethylene | Effects on respiration (indicated by $CO_2$ evolution) |
|---|---|---|
| Control—untreated | 8.09 | Normal respiration, but total rate less than other runs |
| 2% Endothal* | 10.33 | Respiration inhibited for about 2 hr. compared with controls; then stimulated (approximately twice controls) |
| 0.5% trans-cinnamic acid | 8.96 | Respiration inhibited for about 12 hr.; then stimulated (1½ times controls) |
| 2% sodium chlorate-pentaborate | 10.62 | Inhibited respiration temporarily; then greatly stimulated (doubled compared with controls) |

* Disodium 3,6-endoxohexahydrophthalate.

When the plants were presprayed with sodium NA or the stems were placed in sodium NA solution prior to being placed under the bell jars with ethylene, defoliation was severely inhibited (table 3, exp. 2*a*, 2*b*, 3*a*, 3*b*, 3*c*), the latter being the most effective method of application. Neither was the chlorosis of leaves and petioles noted under these conditions. When plants of experiment 3*b* (table 3), previously soaked in 1500-p.p.m. sodium NA for 72 hours, were treated for an additional 48 hours with 100 p.p.m. ethylene, there was essentially no effect (exp. 3*d*, table 3). As indicated in the petiole tests (tables 1, 2), results of experiment 4*a*, 4*b* (table 3) suggest that the relative

the data of table 4 were selected as being the approximate average of each treatment. The results show that in all cases the rate of ethylene evolution was accelerated in the treated leaves. The intimate relationship of ethylene production to the respiratory rate is also demonstrated by the greatly stimulated carbon dioxide evolution in all cases where ethylene production was enhanced. In fact, although not brought out by the data of table 4, the individual runs that showed no significant increase in ethylene production were those with respiratory rates approximately the same as that of the control. CARNS *et al.* (**6**) have reported a positive correlation between the abscis-

sion rate of bean explants and the oxygen level up to 40%. Undoubtedly the influence of oxygen in these experiments is its effect upon aerobic respiration. The necessity of aerobic respiration for ethylene production has also been reported (3, 11, 12).

EVIDENCE ON THE ROLE OF AUXIN IN ETHYLENE PRODUCTION.—From the data of tables 2 and 3 it is seen that applying ten times more ethylene chlorohydrin (1%) than IAA (0.1%) or 200 p.p.m. ethylene gas with 500 p.p.m. sodium NA did not entirely overcome the inhibitory effect of auxin on abscission.

Because of this and in light of previous work (11), it was thought that the role of ethylene in abscission was not solely due to destruction of auxin as suggested by other workers (18, 19). The inhibition of respiration by relatively high amounts of IAA is well known, and it was thought possible that IAA in relatively high amounts also inhibits the production of ethylene by living tissue. Therefore, ethylene evolution from known potent substrates such as pectin and *l*-arabinose (11) was determined in both the presence and the absence of IAA as follows: Two Petri dishes of etiolated pea seedlings were placed above duplicate lots of 500 ml. of 2% pectin or 500 ml. of 1% *l*-arabinose plus 75 ml. of the crude mold-enzyme extract (11) contained in the bottom of separate desiccators. For the pectin substrate 1000 p.p.m. of IAA were added to one lot but omitted from the other. After 72 hours the pea seedlings were severely inhibited in the desiccator containing only the substrate and enzyme source, whereas in the desiccator containing the added IAA growth of the seedlings was comparable with controls. The pectin substrate, however, was cleared and hydrolyzed in both cases. Demonstration that hydrolysis of the pectin was not the limiting factor preventing ethylene production was given by clarification and hydrolysis of this substrate by a commercial pectin-hydrolase, "pectinol A," in either the presence or absence of IAA. However, when using "pectinol A" as the enzyme source, in neither case was ethylene produced as indicated by the etiolated-pea-seedling test. The tests utilizing the arabinose-mold substrate, with and without various concentrations of IAA, are summarized in table 5. Inhibition of the elongation of the pea epicotyls was used as an index of ethylene production by determining their average length before placing in the desiccators with the various substrates and again after 72 hours of exposure. The seedlings were

TABLE 5

INHIBITION OF ETIOLATED PEA EPICOTYLS AS INDEX TO ETHYLENE PRODUCTION FROM 1% ARABINOSE-MOLD EXTRACT SUBSTRATE, WITH AND WITHOUT IAA (MEASUREMENTS IN CENTIMETERS)

| Seedlings above: | Av. initial length of epicotyl | Av. length of epicotyl after 72 hr. | Av. change in length |
|---|---|---|---|
| Water only | 4.5 | 9.0 | 4.5 |
| 1% Arabinose+75 ml. mold extract | 5.0 | 6.0 | 1.0 |
| 1% Arabinose+75 ml. mold extract+10 p.p.m. IAA | 4.5 | 5.0 | 0.5 |
| 1% Arabinose+75 ml. mold extract+100 p.p.m. IAA | 4.0 | 4.5 | 0.5 |
| 1% Arabinose+75 ml. mold extract+500 p.p.m. IAA | 5.0 | 7.5 | 2.5 |
| 1% Arabinose+75 ml. mold extract+1000 p.p.m. IAA | 4.5 | 8.5 | 4.0 |

severely inhibited in the desiccators containing the arabinose-mold extract substrate alone and in those containing 10 or 100 p.p.m. IAA in combination with this substrate, indicating that ethylene was produced under these conditions. Apparently some ethylene was produced in the desiccator containing 500 p.p.m. IAA but very little if any in the desiccator with 1000 p.p.m. IAA.

## Discussion

The auxin-ethylene balance hypothesis as a theoretical explanation for natural abscission was first proposed in concrete form by GAWADI and AVERY (7) without the support of direct experimental evidence. The foregoing data support this hypothesis. Apparently the important factor that prevents young leaves from abscising is their high IAA production. Several investigators (1, 2, 4, 7, 16) have suggested that IAA or other growth-regulators of the auxin type are instrumental in preventing abscission. Others (8, 25) have demonstrated that as leaves mature their hormone supply diminishes and that the auxin gradient across the abscission zone is lost just preceding abscission (25).

It has been shown both in the present work and by others that ethylene or ethylene compounds are capable of inducing abscission even of young immature leaves, presumably high in auxin content. It might therefore be assumed that young leaves are characterized by a relatively high IAA content but low ethylene production. This, however, does not appear to be entirely the case, as it has been observed (11) that young but mature cotton leaves taken from plants at the flowering stage had a higher relative rate of ethylene evolution than older leaves on a weight basis. Young squares and flowers, both under greenhouse and field conditions, have also been observed to be shed easily at this stage. It is suggested that the absolute amount neither of auxin nor of ethylene per se is the important factor that prevents abscission in young leaves but that it may be more likely the auxin gradient across the petiole (26) in relation to ethylene or the relative balance between the two factors, ethylene and auxin. Even though ethylene production is high in young leaves, the ratio of auxin-ethylene is still sufficient to prevent abscission. The present study has shown that either ethylene chlorohydrin or ethylene was very efficient in effecting a high percentage of abscission in either young cotton petioles or mature cotton leaves, but, when such treatment also included 1000 p.p.m. IAA or 500 p.p.m. sodium NA, abscission was greatly reduced. LIVINGSTON (18) has suggested that the role of ethylene as an accelerator of abscission may be due to its destroying action on auxin. In the present work it was demonstrated that a relatively high concentration of IAA inhibited the *in vitro* production of ethylene by the action of enzymes from naturally potent substrates. The suggestion is offered that this is at least partially the effect that auxin exerts in the prevention of leaf-fall under natural conditions. No doubt one of the first effects of artificially supplied ethylene, upon either leaf-abscission processes or the ripening of fruits, is to inactivate or lower the innate auxin of these organs. Then, with the removal of hormone inhibition and further activation of both hydrolytic and respiratory enzymes by ethylene, cellular metabolism becomes greatly accelerated. This was evidenced by the increase in the soluble cell constituents and by enhanced carbon dioxide output. It is believed that these events lead to the exhaustion of the common cellular substrates and ulti-

mately to enzymatic degradation of the more potent ethylene-precursor substrates, such as the pectic compounds of the cell wall (**11**). This explanation accounts for the natural increased evolution of ethylene, as well as for the observation that ethylene functions somewhat paradoxically as an autocatalyst in accelerating its own production. It also harmonizes the suggestion that ethylene destroys auxin (**18, 19**) and that auxin inhibits ethylene production.

In their study of foliar abscission of stored cauliflower and cabbage LEE and CAROLUS (**17**) have also suggested that an effected retardation of foliar abscission was probably due to the interaction of growth-regulating substances with certain enzymatic systems necessary for the initiation of abscission.

The use of an anti-auxin, such as trans-cinnamic acid, gave further evidence that abscission was accelerated in young cotton petioles, supposedly by inactivation of the natural auxin and by predominance of ethylene production which eventually elicited abscission. When the natural auxin content of the petioles was supplemented with applied IAA in conjunction with trans-cinnamic acid, the abscission rate was again reduced. Undoubtedly the role of the commercial defoliants tested, as well as that of other compounds known to promote abscission, is in part due to death or lowered activity of the cells of the leaf and hence to elimination or reduction of IAA synthesis. LIVINGSTON (**18**) arrived at essentially this conclusion in his study of abscission agents. In addition, actual measurement of ethylene production by cotton leaves treated with these compounds and comparison with controls revealed that ethylene output was enhanced under their influence, probably through their effects upon respiration.

Briefly, then, it appears that any factor or combination of factors that disturbs the natural IAA ethylene balance in the leaf either by reducing IAA synthesis or by increasing ethylene evolution leads to the same ultimate end—abscission of the organ regardless of its morphological age.

Granted that the auxin-ethylene balance hypothesis explains adequately the natural cause of defoliation, how then can we reconcile this theory to other factors that also bring about abscission? No attempt will be made to discuss all the known abscission agents, but a few of the better-known factors will be discussed from this viewpoint.

In the case of a change in water relations as the cause of abscission, reference to the literature shows that this can also possibly as logically be interpreted as an indirect cause which exerts its influence by curbing the active auxin content of tissues, by favoring ethylene production, and by regulating turgor forces facilitating separation in the abscission zone. HATCHER (**14**) and others who have followed the IAA content during the development of reproductive structures noted that, associated with desiccation of the fruit and seed during maturation, the measurable active auxin content diminished sharply to a low level. As is well known, however, the free auxin content again rises to a high level following hydration of the seed and renewed activity of the embryo during germination. GOODWIN (**8**) has reported that the amount of diffusible auxin in young *Solidago* leaves is very small but increases to a maximum at the most rapid growth period of the leaf and then falls off with approaching maturity. The moisture content of leaves also follows rather closely these periods of minimal and maximal auxin content. It seems logical that the hydration of

plant tissues is, indirectly at least, intimately associated with their free auxin content. In another investigation (11) it was found that the pentoses, particularly arabinose, serve as excellent sources of ethylene. HARVEY (13) stated that the pentosans can serve as reserve materials when the more readily utilized carbohydrates have been exhausted. He (13) as well as SPOEHR (26) reported that dehydration of tissues favors pentosan formation, which serves as the parent material of the pentoses. As pointed out by BROWN and ADDICOTT (5), water relations are important in abscission, and a rapid change in water relations often precedes leaf-fall in the field. These workers (5) suggested that turgor regulates the tissue tensions and compressions within the abscission zone and facilitates mechanical separation. A condition of water stress in the plant, then, possibly serves a threefold purpose in stimulating abscission: (*a*) by reducing the auxin content, (*b*) by increasing ethylene production, and (*c*) by facilitating mechanical separation during abscission.

Evidence of other workers can also be presented suggesting that temperature, particularly low temperature as a cause of abscission, undoubtedly functions in a similar manner in controlling the auxin-ethylene balance of tissues. HANSEN (12) investigated the effect of temperature on respiration and ethylene production in pear fruits. Between 0° and 20° C. both processes increased; but between 20° and 40° C. carbon dioxide evolution increased steadily, but ethylene production declined sharply, reaching a zero value at 40° C. BIALE and SHEPHERD (3) studied the effects of temperature on the production of ethylene by *Penicillium digitatum* and noted that 14.5° C. was most favorable for formation of the active emanation, whereas production was not apparently increased at 20° or 25° C., while it was inhibited at 2.5° C.

Though much additional evidence has accumulated as to the role of other factors in abscission, indications are that they can also be explained in terms of their effects upon the IAA-ethylene balance of the plant. GUSTAFSON (9) has shown that poor mineral nutritive conditions, low temperature, and high light intensities lower the growth-hormone content of tomatoes and corn, and he stressed that their effect on the physiological status of the plant should be evaluated in interpreting hormone studies. OLMSTED (21) has reported that naturally decreasing autumnal photoperiod basically conditions foliar abscission in the sugar maple and suggested that this might involve shifting gradients of abscission inhibitors and/or activators.

The author is fully aware that much of the foregoing discussion is of a highly speculative nature and leans heavily upon the work of others; but the failure to explain abscission entirely or satisfactorily on the basis of the effect of mineral nutrition, water relations, or chemical composition alone, as well as other environmental factors, lends much support to the auxin-ethylene balance premise.

## Summary

1. A quick petiole test employing the lanolin-paste technique was used to study the role and effectiveness of indoleacetic acid (IAA), ethylene chlorohydrin, trans-cinnamic acid, Shed-A-Leaf (sodium chlorate-pentaborate), and Endothal (disodium 3,6-endoxohexahydrophthalate) in the abscission process. Debladed but attached *Coleus* and cotton petioles were utilized in the study. The defoliation of mature cotton plants by means of ethylene gas, used singly and in various combinations with the sodium

salt of naphthaleneacetic acid (sodium NA), was also investigated.

2. IAA completely inhibited abscission of petioles of both plants, while Endothal and ethylene chlorohydrin induced complete abscission in the case of *Coleus* and a high percentage of abscission for cotton.

3. Trans-cinnamic acid effected a higher percentage of abscission than occurred in the controls (debladed leaves) of both species, indicating the possibility that the compound offsets the inhibitory action of auxin.

4. The more extensive experiments with cotton showed that when Endothal, Shed-A-Leaf, and trans-cinnamic acid (which promoted leaf-fall by themselves) were combined with IAA, the reduction in percentage of abscission was somewhat proportional to the concentration of IAA. However, when ethylene chlorohydrin was combined with IAA or when mature cotton plants were exposed to ethylene gas after pretreatment with sodium NA, the results are more suggestive that it is the relative balance of ethylene to auxin which determines the amount and rate of abscission effected.

5. Determination of the amount of ethylene and carbon dioxide evolved by untreated cotton leaves and comparison with leaves treated with Endothal, trans-cinnamic acid, and Shed-A-Leaf showed a general stimulation in ethylene production by treatment. In all cases ethylene production was closely correlated with carbon dioxide evolution.

6. *In vitro* production of ethylene by action of enzymes on pectin and *l*-arabinose was inhibited by IAA, and it is proposed that this is an important effect of auxin in preventing abscission. It is concluded that artificially supplied ethylene probably inactivates auxin as previously suggested by others, as well as accelerating hydrolase and respiratory enzymes.

7. The data of this study are presented as evidence that the natural cause of abscission is the result of the balance between auxin and ethylene in the petiole. Speculations regarding the role of some of the other factors known to influence the abscission process and reported by other workers are discussed relative to the auxin-ethylene balance hypothesis.

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# EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID UPON THE NITROGEN METABOLISM AND WATER RELATIONS OF SOYBEAN PLANTS GROWN AT DIFFERENT NITROGEN LEVELS[1,2]

S. R. FREIBERG AND H. E. CLARK

## Introduction

A number of investigators have studied the effect of such growth-regulators as indoleacetic acid (**11, 13, 22, 25**), naphthalenacetic acid and naphthalene acetamide (**7, 10**), and 2,4-dichlorophenoxyacetic acid (**9, 17, 19, 21, 24, 28, 29, 30**) on the distribution of nitrogen within plants and have found that these synthetic growth-regulators affect the nitrogen metabolism of plants in a similar manner. The concentrations of total and protein nitrogen, and of most amino acids in these proteins (**19, 28**), generally have decreased in the leaves and in-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Department of Plant Physiology.

[2] This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W-18-064-CM-225 with Rutgers University.

creased in the stems and roots of treated plants.

The effects of indoleacetic acid upon plants grown at different levels of nitrogen nutrition have been reported (**6, 22**). Responses to 2,4-dichlorophenoxyacetic acid by soybean plants grown at different nitrogen levels also have been described (**29**), but no separation of nitrogen fractions was made. Accordingly, an experiment with soybeans grown at two different levels of nitrogen nutrition was carried out in which the distribution in leaves, stems, and roots of several fractions of nitrogen was determined following treatment of the plants with sodium 2,4-dichlorophenoxyacetate (hereafter referred to as 2,4-D) dissolved in the nutrient solution bathing the roots.

## Material and methods

CULTURAL TREATMENT.—Lincoln soybean seeds were germinated in sand on August 3, 1949, and uniform seedlings when approximately 11 cm. tall were transferred to nutrient solutions in gallon glass jars. Three plants were grown in each jar of continuously aerated solution according to the SHIVE and ROBBINS method (**20**). Composition of the nutrient solutions used is shown in table 1.

A nutrient solution at half the concentration of the medium-N solution was used during the first week after the seedlings were transferred. After this, full-strength medium-N solution was used. Twenty-six days after germination twenty-eight cultures of three plants each were placed in a nutrient solution containing no nitrogen (shown as minus-N in table 1); plants grown with this treatment were designated as low-N. Twenty-eight other cultures were placed in a nutrient solution containing a high level of nitrate nitrogen (shown as high-N in table 1) and were designated as high-N plants.

Calcium chloride supplied calcium ions in the minus-N solution, but too high a level of chloride was not desired. Accordingly, the calcium content of the minus-N solution was kept slightly lower than that of the high-N solution. It is not considered that this difference in calcium levels significantly affected the outcome of the experiment.

2,4-D TREATMENT.—At the time of treatment with 2,4-D the plants were 33 days old and had four fully expanded trifoliolate leaves. Low-N plants which had been in solution without nitrogen for 7 days were lighter green in color, and the stems were not nearly so succulent as those of the high-N plants.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS*

| SALT | MOLARITY OF SALTS EMPLOYED | | |
|---|---|---|---|
| | Medium-N | High-N | Minus-N |
| $KH_2PO_4$ | 0.002 | 0.002 | 0.002 |
| $K_2SO_4$ | .001 | | .001 |
| $KNO_3$ | | .002 | |
| $Ca(NO_3)_2$ | .002 | .003 | |
| $CaCl_2$ | .0005 | .0005 | .0025 |
| $MgSO_4$ | .002 | 0.002 | 0.002 |
| $(NH_4)_2SO_4$ | 0.00075 | | |

* Micro-nutrient supplement added to each of above included 1 p.p.m. Fe, 0.5 p.p.m. B, 0.25 p.p.m. Zn, and 0.25 p.p.m. Mn.

At the time of 2,4-D introduction the high-N nutrient solutions were discarded, and nutrient solutions of the minus-N composition were used for all plants. This solution was used during the period of exposure to 2,4-D to prevent marked increases in pH value which result from rapid absorption of the nitrate ion. A pH of 3.9 was used because earlier tests demonstrated that the intensity of response to 2,4-D was greater at this pH level than at higher values (**3, 5**).

The sodium salt of 2,4-D was added at the rate of 4 p.p.m. acid equivalent to the

nutrient solution of twelve cultures at each nitrogen level, this concentration being chosen after preliminary treatment of soybean plants with from 1 to 20 p.p.m. of 2,4-D.

Roots of treated plants were exposed to 2,4-D for 22–23 hours, after which they were rinsed with distilled water, and the high-N plants were again placed in newly prepared high-N nutrient solution of the composition listed in table 1. Low-N plants, after treatment, were placed in a nutrient solution essentially the same as the minus-N solution but containing 14 p.p.m. of nitrate nitrogen to prevent development of excessive nitrogen deficiency.

Control plants at each nitrogen level were harvested just before 2,4-D introduction, and treated and control groups were harvested 1, 4, and 7 days later. Four replicate cultures, each including three plants, were harvested in all cases. Plants were separated into leaf blades (hereafter referred to as leaves), stems and petioles, and roots and rapidly dried in a forced draft oven at 70° C. The dried tissue was then ground in a Wiley mill through a 40-mesh sieve.

ANALYTICAL METHODS.—Total nitrogen was determined on 25 mg. of dried tissue by the micro-Kjehldahl method of PEPKOWITZ and SHIVE (15). Water-soluble nitrogenous constituents were extracted by boiling 0.5 gm. of dried tissue with 35 cc. of distilled water for $3\frac{1}{2}$ minutes. The extracts were then cooled, made to 50 cc. volume, centrifuged, and finally filtered through glass wool. Determinations of total soluble nitrogen, nitrate nitrogen, amide nitrogen, ammonium nitrogen, and alpha-amino nitrogen were made on aliquots of these hot-water extracts.

Aliquots for determination of total soluble nitrogen were placed in 150-mm. Pyrex ignition tubes and evaporated to dryness in an oven at 100° C. Nitrogen content was then determined by the same procedure employed for total nitrogen. To determine nitrate nitrogen, aliquots of the hot-water extracts were analyzed by a modification of the phenoldisulphonic method of HARPER (8), using a Klett-Summerson photoelectric colorimeter instead of Nessler comparison tubes. Total amide nitrogen was determined on aliquots of hot-water extracts after removal of the ammonia according to a procedure used by SCHLENKER (18). Ammonium nitrogen was determined by the vacuum distillation method of PUCHER *et al.* (16). The residue in the still was then analyzed for alpha-amino nitrogen by the nitrous acid VAN SLYKE manometric method (27).

From the analyses made it was possible to calculate total organic nitrogen by subtracting nitrate and ammonium nitrogen from total nitrogen; protein nitrogen, by subtracting soluble nitrogen from total nitrogen; soluble organic nitrogen, by subtracting nitrate and ammonium nitrogen from soluble nitrogen; and residual soluble organic nitrogen, by subtracting amino nitrogen and amide nitrogen from soluble organic nitrogen.

Values from treated and corresponding control plants from each harvest were then submitted to statistical analysis according to SNEDECOR's method (23) of comparison of two samples, and $t$ values for the differences between means beyond the 5% level of probability were designated as significant; those beyond the 1% level of probability were denoted as highly significant.

## Results

VISIBLE PLANT RESPONSES.—Twisting of stem tips and epinasty of petioles were the first noted responses to 2,4-D ap-

plied in the nutrient solution and were observed within $2\frac{1}{2}$ hours. These early signs were then followed by enlargement of stems and bases of petioles, loss of green color from the surface of stems, and wilting and yellowing of leaves. The intensity of response to 2,4-D, however, differed at the two nitrogen levels. High-N soybean plants responded to 2,4-D more rapidly than did low-N plants. Marked curvature of stem tips and epinasty of upper petioles could be seen on high-N plants 3 hours after 2,4-D introduction. Similar signs of response to 2,4-D were not observed on low-N plants until 6 hours after 2,4-D introduction, nor were these symptoms so pronounced as those observed $3\frac{1}{2}$ hours earlier on high-N plants. Progressive development of symptoms proceeded rapidly on high-N plants, which by the end of 7 days (fig. 1) had deteriorated far more than low-N plants.

DRY MATTER CHANGES.—Within 24 hours after start of exposure the percentage dry matter was very significantly lower in leaves of treated plants than in leaves of corresponding controls in both high-N and low-N groups (table 2). Later a rapid deterioration of the high-N plants treated with 2,4-D brought about subsequent wilting and an increase in the percentage of dry matter in their leaves after 4 and 7 days. Stems and petioles as well as roots of the low-N group also showed very significantly lower values of percentage dry matter than did corresponding controls 24 hours after 2,4-D introduction.

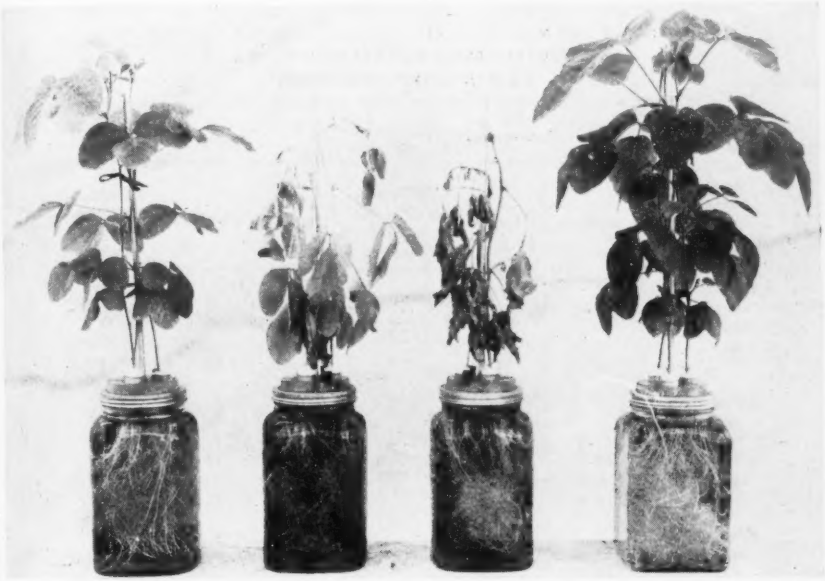

FIG. 1.—Effect of different nitrogen levels on response by soybean plants 7 days after 2,4-D introduction at 4 p.p.m. for 23 hours in nutrient solution. *Left to right:* low-N control; low-N treated; high-N treated; high-N control.

Dry weight yields of tissue fractions

on a per-plant basis are listed in table 3. These values show a steady increase in dry matter with successive harvests in all organs of control plants, in contrast with decreases or at least no significant increases in corresponding organs of treated plants so that by 7 days significant differences between control and treated plants were found in all organs. This was due to the continued growth of the control plants in contrast with no increase in weight of treated plants after exposure to 2,4-D.

CHANGES IN NITROGEN FRACTIONS.—Chemical analyses for nitrogen were made on plant tissue harvested at the time of 2,4-D introduction and 1 and 4 days later. Analyses were not run on tissue harvested at 7 days because of deterioration of the high-N plants by that time. Most leaves of high-N plants were then dry, as shown in figure 1 and in table 2 by the extremely high percentage of dry matter of leaves.

Marked changes in the nitrogenous constituents occurred when plants were treated with 2,4-D. Even though visible responses of the high-N plants to 2,4-D were more rapid and pronounced than those of the low-N plants, changes in nitrogen distribution were similar in both groups. For brevity, only analyses of the high-N plants are reported (table 4).

By 4 days after 2,4-D introduction the percentages of total-N, organic-N, and nitrate-N were very significantly lower in leaves of treated plants than in controls harvested at that time, and protein-N was significantly lower in treated than in control leaves. At the same time the con-

TABLE 2

PERCENTAGE DRY MATTER IN SOYBEAN PLANTS GROWN ON HIGH- AND LOW-NITROGEN LEVELS AND TREATED WITH 4 P.P.M. 2,4-D IN NUTRIENT SOLUTION

| Days after treatment | Low-nitrogen | | | High-nitrogen | | |
|---|---|---|---|---|---|---|
| | Controls | Treated | Significance of difference between means[a] | Controls | Treated | Significance of difference between means[a] |
| *Leaves:* | | | | | | |
| 0 | 19.8 | | | 16.6 | | |
| 1 | 20.1 | 18.6 | ** | 17.0 | 15.8 | ** |
| 4 | 20.1 | 16.9 | ** | 16.1 | 21.0 | ** |
| 7 | 20.8 | 24.4 | * | 17.8 | 68.3 | ** |
| *Stems and petioles:* | | | | | | |
| 0 | 16.5 | | | 11.8 | | |
| 1 | 17.5 | 16.5 | ** | 12.4 | 12.0 | † |
| 4 | 19.3 | 13.6 | ** | 11.4 | 10.6 | * |
| 7 | 19.9 | 14.0 | ** | 12.5 | 13.5 | † |
| *Roots:* | | | | | | |
| 0 | 11.3 | | | 8.4 | | |
| 1 | 12.1 | 11.5 | ** | 9.4 | 9.3 | † |
| 4 | 11.3 | 11.3 | † | 9.0 | 9.4 | † |
| 7 | 9.4 | 10.4 | † | 7.8 | 9.6 | * |

[a] Significance level of probability indicated: ** beyond 1%; * beyond 5%; † less than 5%.

centrations of soluble organic-N, alpha-amino-N, amide-N, and residual soluble organic-N were very significantly higher in the leaves of treated plants than in those of the corresponding controls. In the stems and petioles percentages of total-N, organic-N, protein-N, soluble organic-N, and residual soluble organic-N were all very significantly higher in treated plants than in controls. Only nitrate-N showed a very significantly lower concentration in stems and petioles of treated plants than in corresponding controls on the fourth day. In the roots total-N, organic-N, and residual soluble organic-N were at least significantly higher in treated plants than in controls when expressed on a percentage-dry-weight basis. Again, the concentration of nitrate-N was very significantly less in treated plants than in controls.

At the end of the 1-day exposure period the nitrate-N content of all organs of control and treated plants was considerably less than at the start of treatment. This sharp decline of nitrate-N in both control and treated plants when no nitrate-N was included in the nutrient solution during the 23-hour treatment period appears to reflect the rapid utilization of this form of nitrogen by the soybean plant.

TABLE 3

TOTAL DRY WEIGHT (GM. PER PLANT) OF TISSUE FRACTIONS FROM SOYBEANS GROWN ON HIGH- AND LOW-NITROGEN LEVELS AND TREATED WITH 4 P.P.M. 2,4-D IN NUTRIENT SOLUTION

| | Low-nitrogen | | | High-nitrogen | | |
|---|---|---|---|---|---|---|
| Days after treatment | Controls | Treated | Significance of difference between means[a] | Controls | Treated | Significance of difference between means[a] |
| *Leaves:* | | | | | | |
| 0 | 0.70 | | | 1.01 | | |
| 1 | 0.71 | 0.80 | † | 0.97 | 1.08 | † |
| 4 | 0.83 | 0.71 | † | 1.42 | 0.98 | * |
| 7 | 0.98 | 0.65 | ** | 2.09 | 0.94 | ** |
| *Stems and petioles:* | | | | | | |
| 0 | 0.42 | | | 0.52 | | |
| 1 | 0.45 | 0.55 | † | 0.48 | 0.58 | † |
| 4 | 0.57 | 0.58 | † | 0.73 | 0.62 | † |
| 7 | 0.71 | 0.60 | * | 1.00 | 0.61 | * |
| *Roots:* | | | | | | |
| 0 | 0.32 | | | 0.39 | | |
| 1 | 0.33 | 0.35 | † | 0.38 | 0.35 | † |
| 4 | 0.46 | 0.42 | † | 0.56 | 0.35 | ** |
| 7 | 0.65 | 0.38 | ** | 0.77 | 0.29 | ** |
| *Total plant:* | | | | | | |
| 0 | 1.44 | | | 1.92 | | |
| 1 | 1.49 | 1.70 | † | 1.83 | 2.01 | † |
| 4 | 1.86 | 1.71 | † | 2.71 | 1.95 | * |
| 7 | 2.34 | 1.63 | ** | 3.86 | 1.84 | ** |

[a] Significance level of probability indicated: ** beyond 1%; * beyond 5%; † less than 5%.

Even though nitrate-N was again included in all nutrient solutions after the 2,4-D-exposure period, the amount of nitrate-N in each organ of the treated plants was considerably below that in the corresponding organs of controls 4 days after 2,4-D introduction. This presumably was associated with a lower rate of absorption of nitrate-N by treated plants than by control plants, although the same result could have occurred if the assimilation rate of nitrate-N was higher in treated plants than in controls.

To determine whether the accumulation of organic nitrogen in stems and roots of treated plants resulted from an absorption of nitrate by the roots with subsequent assimilation or from a trans-

TABLE 4

NITROGEN FRACTIONS IN HIGH-N PLANTS EXPRESSED AS PERCENTAGES OF DRY WEIGHT OF TISSUE HARVESTED AT INTERVALS AFTER TREATMENT WITH 4 P.P.M. 2,4-D IN NUTRIENT SOLUTION

| Nitrogen fraction | Days after treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 days | 1 day | | | 4 days | | |
| | Controls | Controls | Treated | Significance of difference between means[a] | Controls | Treated | Significance of difference between means[a] |
| *Leaves:* | | | | | | | |
| Total-N | 5.59 | 5.25 | 5.42 | † | 5.08 | 4.65 | ** |
| Organic-N | 5.40 | 5.22 | 5.38 | † | 4.99 | 4.60 | ** |
| Protein-N | 4.52 | 4.42 | 4.34 | † | 4.12 | 3.50 | * |
| Soluble organic-N | 0.88 | 0.80 | 1.04 | ** | 0.87 | 1.11 | ** |
| Alpha-amino-N | 0.28 | 0.19 | 0.26 | ‡ | 0.31 | 0.39 | ** |
| Amide-N | 0.07 | 0.06 | 0.07 | * | 0.07 | 0.11 | ** |
| Residual soluble organic-N | 0.53 | 0.55 | 0.71 | ‡ | 0.49 | 0.61 | ** |
| Nitrate-N | 0.18 | 0.02 | 0.03 | † | 0.08 | 0.03 | ** |
| Ammonium-N | 0.01 | 0.01 | 0.01 | ‡ | 0.01 | 0.02 | † |
| *Stems and petioles:* | | | | | | | |
| Total-N | 3.20 | 2.69 | 2.99 | * | 2.82 | 3.71 | ** |
| Organic-N | 2.62 | 2.30 | 2.69 | ** | 2.36 | 3.60 | ** |
| Protein-N | 1.89 | 1.78 | 1.91 | † | 1.72 | 2.52 | ** |
| Soluble organic-N | 0.73 | 0.52 | 0.79 | ** | 0.64 | 1.08 | ** |
| Alpha-amino-N | 0.21 | 0.17 | 0.15 | ‡ | 0.20 | 0.25 | † |
| Amide-N | 0.11 | 0.06 | 0.09 | ** | 0.09 | 0.09 | † |
| Residual soluble organic-N | 0.41 | 0.29 | 0.55 | ‡ | 0.35 | 0.74 | ** |
| Nitrate-N | 0.57 | 0.38 | 0.29 | * | 0.45 | 0.09 | ** |
| Ammonium-N | 0.01 | 0.01 | 0.01 | ‡ | 0.01 | 0.02 | † |
| *Roots:* | | | | | | | |
| Total-N | 3.91 | 3.04 | 3.66 | ** | 3.26 | 3.67 | ** |
| Organic-N | 3.09 | 2.95 | 3.57 | ** | 2.72 | 3.53 | ** |
| Protein-N | 2.53 | 2.45 | 2.96 | ** | 2.23 | 2.84 | * |
| Soluble organic-N | 0.56 | 0.50 | 0.61 | † | 0.49 | 0.68 | ** |
| Alpha-amino-N | 0.15 | 0.10 | 0.12 | ‡ | 0.10 | 0.12 | † |
| Amide-N | 0.12 | 0.11 | 0.10 | † | 0.13 | 0.14 | † |
| Residual soluble organic-N | 0.29 | 0.29 | 0.39 | ‡ | 0.26 | 0.42 | ** |
| Nitrate-N | 0.81 | 0.07 | 0.07 | † | 0.53 | 0.12 | ** |
| Ammonium-N | 0.01 | 0.02 | 0.02 | ‡ | 0.01 | 0.02 | † |

[a] Significance level of probability indicated: ** beyond 1%; * beyond 5%; † less than 5%; ‡ no statistical analysis made on composited samples.

location of nitrogen from the leaves, these nitrogen fractions were expressed on a per-plant basis. Values in table 5 indicate that no significant amount of nitrate nitrogen was absorbed by the plants after treatment with 2,4-D, because no increase in total nitrogen was of the controls which were much smaller than in the other replicates.

Although many differences in table 4 between control and treated organs were highly significant when the distribution of nitrogen was expressed on a percentage basis, similar differences were not always

TABLE 5

TOTAL, ORGANIC, AND NITRATE NITROGEN IN HIGH-N PLANTS EXPRESSED AS MILLIGRAMS OF NITROGEN PER PLANT IN TISSUE FRACTIONS OF SOYBEANS HARVESTED AT INDICATED INTERVALS AFTER EXPOSURE OF ROOTS TO 4 P.P.M. 2,4-D IN NUTRIENT SOLUTION

| Nitrogen fraction[a] | Days after treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 days | 1 day | | | 4 days | | |
| | Controls | Controls | Treated | Significance of difference between means[b] | Controls | Treated | Significance of difference between means[b] |
| *Leaves:* | | | | | | | |
| Total-N | 56.46 | 50.82 | 58.48 | † | 72.07 | 45.34 | * |
| Organic-N | 54.68 | 50.61 | 58.18 | † | 71.00 | 45.10 | * |
| Nitrate-N | 1.78 | 0.21 | 0.30 | † | 1.07 | 0.24 | ** |
| *Stems and petioles:* | | | | | | | |
| Total-N | 16.52 | 12.91 | 17.16 | † | 20.52 | 22.85 | † |
| Organic-N | 13.58 | 11.09 | 15.52 | * | 17.26 | 22.27 | † |
| Nitrate-N | 2.94 | 1.82 | 1.64 | † | 3.26 | 0.58 | † |
| *Roots:* | | | | | | | |
| Total-N | 15.23 | 11.55 | 12.81 | † | 18.22 | 12.91 | ** |
| Organic-N | 12.08 | 11.28 | 12.57 | † | 15.25 | 12.50 | ** |
| Nitrate-N | 3.15 | 0.27 | 0.24 | † | 2.97 | 0.41 | ** |
| *Total plant:* | | | | | | | |
| Total-N | 88.21 | 75.28 | 88.45 | † | 110.81 | 81.10 | * |
| Organic-N | 80.34 | 72.98 | 86.27 | † | 103.51 | 79.87 | † |
| Nitrate-N | 7.87 | 2.30 | 2.18 | † | 7.30 | 1.23 | ** |

[a] Organic-N includes ammonium-N which did not exceed 0.4 mg. in total plant.

[b] Significance level of probability indicated: ** beyond 1%; * beyond 5%; † less than 5%.

obtained. In contrast, by 4 days control plants had accumulated a significantly greater amount of nitrogen than treated plants. The lack of a significant difference in the organic-N fraction between control and treated plants harvested on the fourth day is probably due to variability introduced by the plants in one replicate evident when comparisons were made on the basis of milligrams of nitrogen per organ per plant. This was because controls weighed more than treated plants by the fourth-day harvest. The total amount of a constituent in a control organ might then be greater than in the treated one even though the concentra-

tion of the same fraction might be lower in the control. Accordingly, no further analysis of data calculated on a per-plant basis is reported.

## Discussion

Visible symptoms of 2,4-D treatment were much more pronounced in high-N than in low-N soybean plants. WOLF *et al.* (29) also observed a greater response to 2,4-D by high-N than by low-N soybean plants in an earlier experiment involving a different technique for application of 2,4-D in the nutrient solution. Similar differences between high-N and low-N plants were also observed by GOLDBERG (6) in cabbage plants treated with indole(3)acetic acid. No explanation can be given for this difference in response to 2,4-D except to suggest that following 2,4-D treatment high-N plants showed greater tendency toward epinasty and twisting because of their more succulent tissues, whereas low-N plants were much less succulent and accordingly would withstand epinasty and twisting of stems better than high-N plants.

Several hypotheses might be advanced for the early highly significant decreases in percentage dry weight of tissue fractions, particularly of leaves of treated plants. It is recognized that 2,4-D might have stimulated respiration and thereby caused some loss of solids from the tissues of treated plants over a period of time (2, 29), which would then account for the decrease in the percentage dry weight. Quantitatively, it is not considered likely that loss of respirable dry matter by the treated plants would be sufficient to cause such a marked decrease in percentage dry matter within 24 hours. No decrease in percentage dry matter of treated plants was found in another experiment when comparable soybean plants were harvested on a very humid day at the end of 24 hours' exposure to 2,4-D.

Accordingly, it appears that treatment with 2,4-D has an early effect upon the water relations of tissues. Whether this is due to some effect of 2,4-D upon cytoplasmic hydration (4), osmotic concentrations of cells (12), or degree of opening of stomata (1), or a combination of these factors has not been established in our experiments. A similar early decrease in percentage dry matter of treated plants has been obtained a number of times with the nasturtium as well as with the soybean. This phenomenon probably deserves further attention. It should be pointed out, however, that treated plants showing this early decrease in percentage dry matter—accompanied by obvious crispness and turgidity of leaves—later wilted and increased markedly in percentage of dry matter.

When a higher concentration of 2,4-D was used in other experiments, plants wilted and leaves dried much earlier without the initial phase of higher water content. Under such conditions roots of treated plants appeared water-soaked within 24 hours. Also with high concentrations of 2,4-D active water absorption by the roots was apparently greatly reduced, as evidenced by lack of bleeding from cut stumps or absence of guttation (by corn) from intact treated plants when corresponding untreated control plants were bleeding or guttating freely.

It appears that either an increase or a decrease in water-holding capacity of tissues can result from treatment with 2,4-D, depending upon the relative concentration acting on the tissues. CURRIER (4) has noted reactions of cells of several different plants to increasing concentrations of 2,4-D and other herbicides which

were interpreted on the basis of a stimulation of cytoplasmic hydration by low concentrations and a retardation at higher concentrations. In this respect the water economy of the cell and that of the plant as a whole show the same phases of stimulation at low concentrations of 2,4-D and retardation at high concentrations characteristic of a number of other responses by plants to auxins (**26**).

In general the results obtained indicate that 2,4-D is responsible for a marked decline in the protein nitrogen in leaves of treated plants. The concentration of total-N per unit of tissue was 9% less in treated than in control leaves 4 days after 2,4-D introduction. The loss appears to be accounted for by the increase in protein and other forms of nitrogen in the stems of treated plants and to a lesser extent in the roots.

The inhibition of nitrate accumulation by treated roots has also been noted when excised wheat roots were exposed to 2,4-D (**14**). Thus, direct exposure to 2,4-D appears to change the absorbing capacity of roots. Failure of treated soybean plants to accumulate nitrate in the tops is in direct contrast to the findings of STAHLER and WHITEHEAD (**24**). They found a marked increase in nitrate in leaves of sugar-beet plants treated with 2,4-D. However, these plants were sprayed with 2,4-D, and perhaps the roots were not injured to the extent of limiting absorption.

The increased protein-N in the stems and petioles of treated plants appears to result from translocation from the leaves of the protein-N which has been hydrolyzed to a soluble form. In all organs of treated plants the soluble organic-N was higher than in the controls 1 day and 4 days after 2,4-D introduction, more than half of the increase in soluble organic-N being accounted for by the residual soluble organic-N fraction. Presumably, the increase in soluble organic nitrogen in the treated leaves is due to the breakdown of protein into a soluble form, whereas the increase in the stems and roots is due to soluble nitrogen translocated from the leaves. This conclusion seems to be justified because of the presence of greater amounts of protein in the stems and roots of treated plants than of controls. These findings appear to be in accord with those of SELL *et al.* (**19**) and WELLER *et al.* (**28**), who also found a loss of protein from the leaves of plants treated with 2,4-D and a marked increase of protein in the stems of treated plants.

The stimulation of protein accumulation in stems which accompanies translocation of nitrogen to the stems from the leaves suggests that 2,4-D affects the activity of proteolytic enzymes. MITCHELL and STUART (**13**) discussed this possibility in connection with nitrogen changes in the hypocotyls of bean cuttings treated with indoleacetic acid. Investigations are now under way to determine how 2,4-D affects the proteases in the several organs of the soybean plant.

## Summary

1. High- and low-nitrogen soybean plants grown in nutrient solutions were treated by introduction of sodium 2,4-dichlorophenoxyacetate (2,4-D) in the nutrient solutions at a concentration of 4 p.p.m. of the acid equivalent. After exposure to 2,4-D for 22–23 hours the plants were placed in newly prepared solutions similar to those used before the treatment. Harvests were made at the start of 2,4-D treatment and 1, 4, and 7 days later. Plants of each group were divided into leaf, stem and petiole, and root fractions, and fresh and dry weights

of each fraction were taken. Analyses for total, soluble, amide, alpha-amino, ammonium, and nitrate nitrogen were made on the tissue of each fraction. Changes in distribution of the different forms of nitrogen in the organs following treatment were similar for both high-N and low-N plants. For brevity, the nitrogen analyses for the high-N plants only are reported.

2. Twisting of stem tips and epinasty of petioles, later followed by yellowing of leaves and swelling of stems and petiole bases, and ultimately by wilting and drying of leaves, were visible responses to 2,4-D treatment and appeared sooner in high-N than in low-N plants.

3. Neither high-N nor low-N plants showed any appreciable change in total dry weight after exposure to 2,4-D, in contrast with respective untreated control plants which continued to accumulate dry matter.

4. By the end of the 23-hour period following the start of exposure to 2,4-D, the percentage dry matter of leaves of treated plants was very significantly less than that of corresponding controls. Some days later, however, leaves of the treated plants wilted and showed a much higher percentage of dry matter. Possible factors affecting the water relations of the treated plants have been mentioned.

5. Treatment with 2,4-D apparently changed the absorbing capacity of roots as indicated by failure of treated plants to increase their content of total nitrogen, including nitrates, after exposure to 2,4-D.

6. Plants treated with 2,4-D showed a decrease in the percentage of protein nitrogen in the leaves and an increase in the concentration of this fraction in stems and roots as compared with untreated controls. At the same time, all organs of treated plants showed increases in soluble organic nitrogen fractions, particularly in undetermined forms classed as residual soluble organic nitrogen, over corresponding controls. Apparently some of the protein in the leaves of treated plants was hydrolyzed, and the products were translocated to the stems and roots where more protein was resynthesized. These results suggest that 2,4-D may affect proteolytic enzymes differently in leaves than in stems or roots.

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# AN ATYPICAL GROWTH OF CABBAGE-SEEDLING ROOTS. I. MORPHOLOGY, HISTOLOGY, AND INDUCTION CONDITIONS[1]

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## Introduction

Atypical growths of various plant parts are by now familiar objects of study in the botanical sciences. Such growths can be induced by biological, genetic, mechanical, or chemical factors (cf. review by P. R. WHITE [48]). The present work is concerned with an atypical growth of the roots of cabbage seedlings grown under sterile conditions in the presence of the synthetic growth substance para-chlorophenoxyacetic acid (POA).

The work was initiated following preliminary experiments on the effect of POA on cabbage leaf disks grown in sterile culture. The leaf disks were cultured according to the method of DE ROPP (13); and POA was added to the medium in a concentration range encompassing growth effects from stimulatory to inhibitory. When yeast extract was added to the medium together with POA at an inhibitory concentration, callus-like proliferations were produced along the cut edges of the disks; these proliferations did not appear on the disks when either POA or yeast extract was used alone. The component of the yeast extract responsible for this callus-stimulating activity thus appeared to be of considerable interest, since knowledge of the factor might provide a clue either to the chemical mechanism of action of POA or to the chemical factors involved in the production of such calluses, or both.

In the search for a simpler, more reproducible assay for the yeast-extract factor, it was observed that massive and tumorous proliferations occurred on the roots of cabbage seedlings grown in the presence of POA plus yeast extract and that these growths appeared to a much lesser extent with POA alone. Such growths have been studied in detail, and the work with leaf disks was discontinued. In this paper the morphology and histology of these atypical growths of the roots of cabbage seedlings are described, together with the conditions affecting their induction.

## Material and methods

Cabbage seeds (*Brassica oleracea* var. Copenhagen Market) were surface sterilized by treatment with 10% sodium hypochlorite for 45 minutes and then planted in Petri plates for germination on sterile distilled water agar. Germination was permitted to proceed until a majority of the seedlings had radicles 5 mm. long. Lots of five each were then removed and placed in 50-ml. Erlenmeyer flasks, each containing 20 ml. of the medium to be tested. Growth on the medium continued in darkness at 25° C. for 12–14 days, by which time the pertinent morphological responses had appeared very clearly.

[1] This work embodies a portion of a thesis submitted to the Department of Plant Science of Yale University by the senior author, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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The growth medium had as its basal content (hereafter referred to as B) 1% agar, 2% sucrose, a nutrient salt solution (BONNER's for radish-root growth *in vitro*—see WHITE [47:105]), and a complete vitamin mixture (*Neurospora* complete—see BEADLE and TATUM [4]).

For histological preparation the tissues were excised from the seedlings, rinsed vigorously to remove bits of agar, and fixed in a chromacetic solution containing 0.7 gm. chromic acid, one drop of acetic acid, and 99 ml. distilled water. They were then dehydrated through an alcohol series, impregnated with chloroform, and imbedded in paraffin. Microtome sections cut at 12 $\mu$ were stained with basic fuchsin (1 gm. basic fuchsin, 10 ml. 95% alcohol, 5 gm. phenol, 100 ml. distilled water). Sections were mounted in Canada balsam and photographed (at varying magnifications) using a green filter.

## Results

### GROSS MORPHOLOGY

Figures 6–8 are photographs of representative atypical growths. The morphology of tissue formed from the primary root of cabbage seedlings placed in contact with POA plus yeast extract is actually quite variable; and with intermediate concentrations of POA plus yeast extract many intermediate stages are often seen between typical root morphology and the most modified appearance. The most strongly affected roots have masses of disorganized tissue occurring along all points of the circumference of the primary root; however, this is in the unfavorably high range of POA, and such tissue does not grow well or extensively. Somewhat lower concentrations of POA induce two or more rows of curved and arched plates growing out along the length of the primary root, giving the appearance of winglike extensions. These may grow in a single arch or curve or may break into two or more separating plates initiating growth in several directions. The plates may be thin or massive and may extend along the entire length of the primary root or be localized to particular areas. Less strongly affected primary roots produce restricted cylindrical or platelike outgrowths similar to those reported by ZIMMERMAN and HITCHCOCK (52).

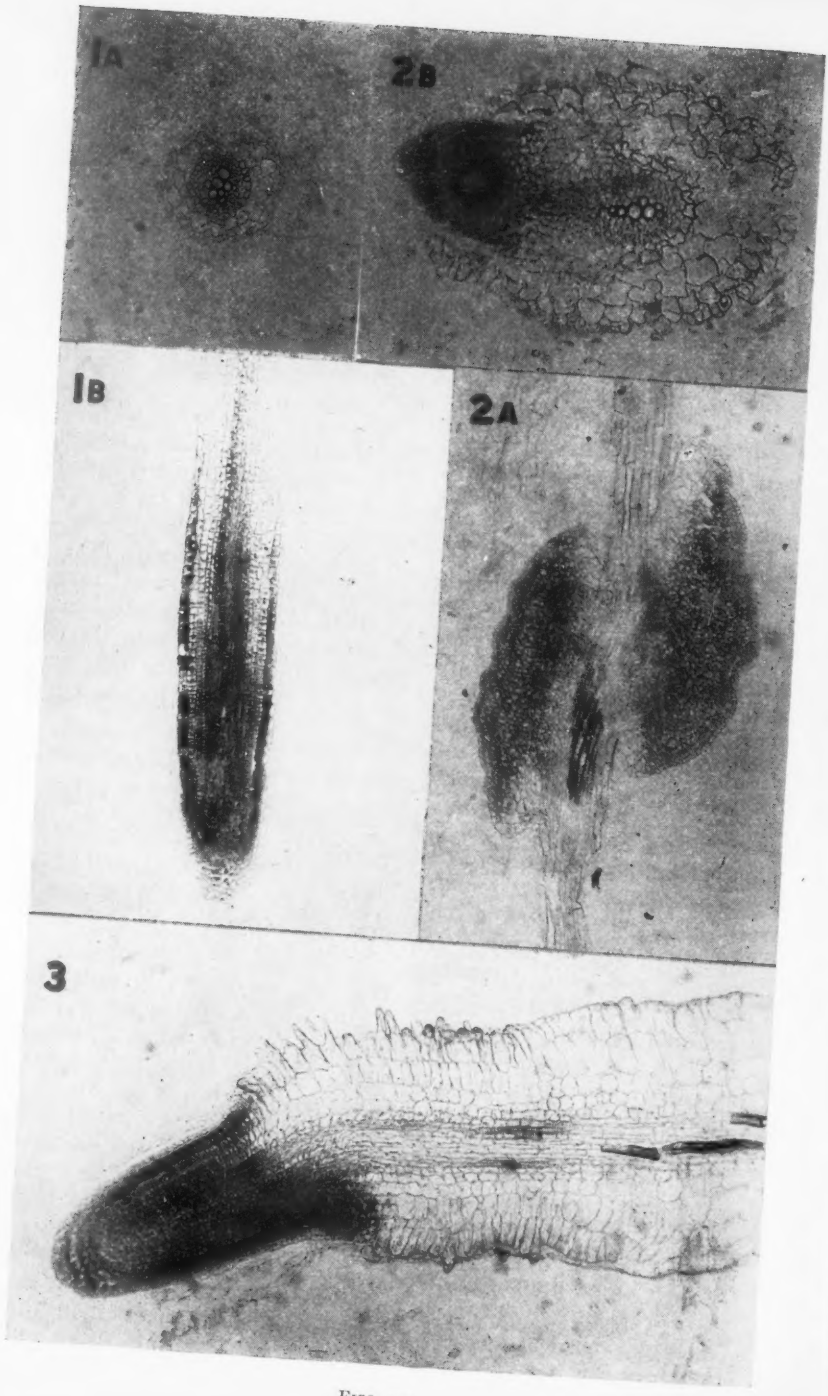
Where a primary root is not producing lateral outgrowths, it has a tendency to be thickened and irregularly curled. The region immediately in back of a primary-root meristem, already established at the time of contact with POA, after further growth is almost invariably found to be spread out into a ridge or shoulder for a little distance; this ridge is flat and is reduced to the dimensions of the rest of the primary root by a gradual curve.

### HISTOLOGY

Histological preparations were made of normal roots and of roots which had developed atypical outgrowths after 3, 7, and 14 days' growth in the presence of POA plus cofactor.

Sections of the normal cabbage-seedling root are shown in figure 1*A*, *B*. Figure 1*A* is a cross section of the root, showing an early stage of the diarch stele; pericycle, endodermis, cortex, and epidermis are already present. A longitudinal section of the terminal meristem and of the region of cell elongation is shown in figure 1*B*.

A longitudinal section of a root that had been growing for 3 days in the presence of POA before sectioning is shown in figure 2*A*. Massive outgrowths arise on either side of the primary root. Although it appears that a single large and flat-




FIGS. 1–3.

tened meristematic ridge is present, it should be pointed out that the section does not pass the center of the original primary root. Thus the edges of the masses on either side are probably not the median portions of the meristems and may represent several closely connected, merging root tips.

Figure 2*B* is of a section perpendicular to the axis of the original root from a piece of material very similar to that illustrated in figure 2*A*. The abnormal growth is seen to arise from the pericylic region, opposite one of the two protoxylem points, and then push its way out through the cortex to the surface of the root in a manner similar to secondary roots. In all such cross sections as figure 2*B* it must be remembered that the meristematic region seen is not actually conical like a normal root meristem but continues up and down perpendicularly to the plane of the photograph, giving rise to the observed ribbon or platelike structure.

Production of the abnormal growth is seen to involve activity of the pericycle and possibly of the endodermis. Xylem, cortex, and epidermis of the primary root are inactive in the original formation of the atypical growth. Cortical and epidermal tissues are split aside by the new growth (and split on the other side of the root by a similar outgrowth not seen in this section).

The marginal region of a cross section of a root system grown for 7 days in the presence of POA is shown in figure 3. The cut was identical with that of figure 2*B* (i.e., perpendicular to the primary-root axis), but only the outer edge of one platelike outgrowth occurring on one side is shown. By this time the atypical growth has assumed its predominant morphological features. The meristem seen is actually a cross section of a ribbon-like meristematic region. Note, however, the vascularization and organization typical of roots. A root cap and a meristem with histogen-like layers are clearly discernible. Scalariform tracheids are differentiated in the central region (again, actually the middle region of a platelike formation), and these appear a relatively short distance from the meristem. An endodermis, with Casparian strips, and a pericycle are distinguishable in this section.

Perhaps the outstanding characteristic of this material is the very rapid expansion of cells immediately to the rear of the meristematic region. Within a very short distance the cells become large and vacuolate; and very few cells are seen in the intermediate stages. This is especially noticeable for cells on the surface, which instead of elongating in a direction parallel to the axis of growth (which is away from the primary root) are distinctly hypertrophied perpendicu-

FIGS. 1–3.—Fig. 1*A*, cross section of normal cabbage-seedling root, showing epidermis, cortex, endodermis, pericycle, and primary xylem. ×90. Fig. 1*B*, longitudinal section of normal root tip. Root cap, meristem, and histogens are all visible. Section includes cell-enlargement stage. ×80. Fig. 2*A*, longitudinal section of primary root showing tumorous outgrowths occurring laterally after 3 days of growth. The cut is probably not through middle of root, so edges of tissue masses are probably not the actual meristems. Scalloping of edges indicates that several discrete meristems may be involved in growth of this tissue. ×75. Fig. 2*B*, cross section of atypical root grown for 3 days on POA and ammonium chloride. Atypical, tumorous tissue is pushing out at side. Proliferation of pericycle and possibly of endodermis is involved in formation of tumor, and cortex is split during its growth. ×130. Fig. 3, outer margin of cross section of primary root grown for 7 days in presence of POA and ammonium chloride. Original primary root is not visible in picture, but only the outer edge of a tumorous plate. Differentiation of central stele with scalariform tracheids, xylem parenchyma, pericycle, and endodermal cells with Casparian strips occurs a short distance from meristem. Atypical features include short distance in which cell enlargement and elongation take place, hypertrophy of outer cortical and epidermal cells, and papillate epidermal cells with swollen bases. ×110.

lar to the surface and appear to form almost a palisade layer. Similarly hypertrophied epidermal cells were illustrated by BOND (8) of pea roots treated with 2,4,5-trichlorophenoxyacetic acid. Cells with papillae near the meristem possess swollen bases and may not even be analogous to root-hair cells in that no unequal division figures are seen.

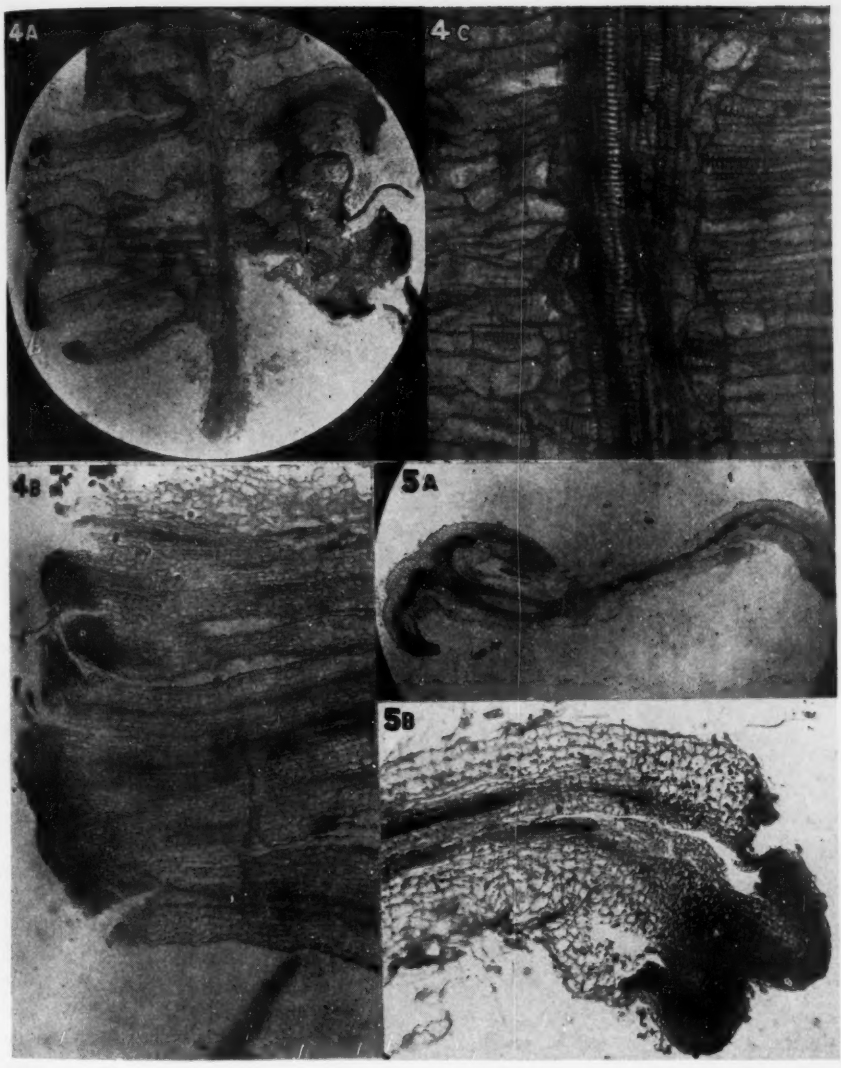
A longitudinal section of a root grown for 14 days on POA and ammonium chloride is shown in figure 4*A*; the atypical growth (in this system relatively flat) has pushed out on both sides. It is evident that the meristematic region of this atypical growth is definitely localized and, at least in a general way, rib shaped. Figure 4*B*, however, is a higher magnification of another section similar to that shown in figure 4*A*, and in it local regions of the meristem are seen to be rounded up apart from the rest of the rib and to constitute individual root meristems. Also, in both figures individual vascular traces can be observed to connect with the meristematic region. It would seem, therefore, that the meristematic regions consist both of meristematic ridges and, in local areas, of individual root-tip meristems. There is some uncertainty in this conclusion, though, since it is not entirely clear whether the regions which appear to form ridges may not actually be regions in back of individual single meristems. The appearance of a ridge may then be an artifact of the particular angle at which the section was cut.

The intimate connections of vascular elements of the atypical material with the stele of the primary root are apparent in a higher magnification (fig. 4*C*) of the central region of figure 4*A*.

A cross section of a root system grown on POA for 14 days is pictured in figure 5*A*. Well-developed plates have formed to either side. This section, comparable with that of the fasciated aerial root of tropical grape illustrated by ZIMMERMANN and HITCHCOCK (52), shows a much more advanced state of proliferation. The morphological characteristics of the atypical growth noted in figure 3 may be observed at various points, especially in the region resembling the ends of an anchor. It is obvious that the growth to the left originated as two closely appressed meristems starting on only slightly different radii on the primary root, which grew in conjunction for a distance and then finally split apart. A photograph of a section further up on the same primary root is shown in figure 5*B*. At this point two lines of meristems on the right side have not yet separated, and the tissues laid down by them are continuing to exist as a single extra-wide plate. From these photographs it can be seen that the atypical proliferation may be formed by meristems that adjoin in more than one dimension. The capacity for massive growth becomes more understandable, since in this case the observed plate was laid down by a line of meristems two layers deep, which only later, and in certain sections, split apart.

From these observations it may be concluded that the atypical growth consists primarily of lateral root tissue, growing in appressed and nondivergent fashion, and distinguishable as separate roots only on the basis of histological features. The growth rates of roots in the mass or plate are synchronized, and the nondivergent state is maintained.

It is apparent from the amount of differentiation to be seen in this material that it is not analogous to the type of tissue usually designated as a callus (for pictures of typical calluses see WHITE [47]). It is better described as a fasciated and comparatively highly organized root




FIGS. 4, 5.—Sections of tumors grown for 14 days on POA and ammonium chloride. Fig. 4*A*, longitudinal. Extensive fasciated plates of tissue have grown out on either side of primary root. Separate vascular traces are distinguishable, laid down in back of riblike meristematic regions. ×15. Fig. 4*B*, enlargement of marginal region of section similar to that in fig. 4*A*. An apparent meristematic ridge is present, together with two individual root meristems. Individual vascular traces are in evidence. ×48. Fig. 4*C*, enlargement of central region of section similar to that in fig. 4*A*. Notice extensive vascular elements of fasciated plates of tumorous tissue, growing out laterally from vascularized primary root in center, and intimate connections between the two. ×196. Fig. 5*A*, cross section of primary root, showing extensive tumor formation on either side. Notice tendency of edges of plates to point down. Two closely adjoining vascular traces on left side indicate that two meristems at points of "anchor" formation must have been growing in intimate connection at earlier stage. ×19. Fig. 5*B*, outer margin of plate of tumorous tissue. Two atypical meristems are here seen in fasciated condition. These two are continuous with others perpendicular to plane of page, so that connation has occurred in two dimensions. In sections made farther along on same tissue, only one meristem is in evidence (right side of fig. 5*A*). Separation of two meristems such as these is undoubtedly responsible for "anchor" formation seen on left side of figure 5*A*. ×93.

proliferation. The term "fasciation" is used according to the definition given by BAUSOR (**2**:383): "Fasciation is an abnormal condition resulting in the flattening of normally cylindrical structures." In the present instance the normally cylindrical structures are the lateral roots, which are found in atypical, nondivergent condition, leading to the formation of a more or less flattened structure.

Since the atypical growth is induced by a synthetic growth substance and requires the continued presence of the growth substance for maintenance of its atypical form of growth (JAGENDORF and BONNER, unpublished data), it cannot be called a tumor in the usual sense of a self-maintaining, "malignant" type of growth. The term "tumor," however, has been applied by many investigators to similarly hormone-induced and dependent growths which are relatively highly differentiated (**18, 21,** etc.). For the sake of convenience, therefore, and in view of this precedent, the atypical root proliferation will be called a "tumor" in this series of papers.

## INDUCTION CONDITIONS

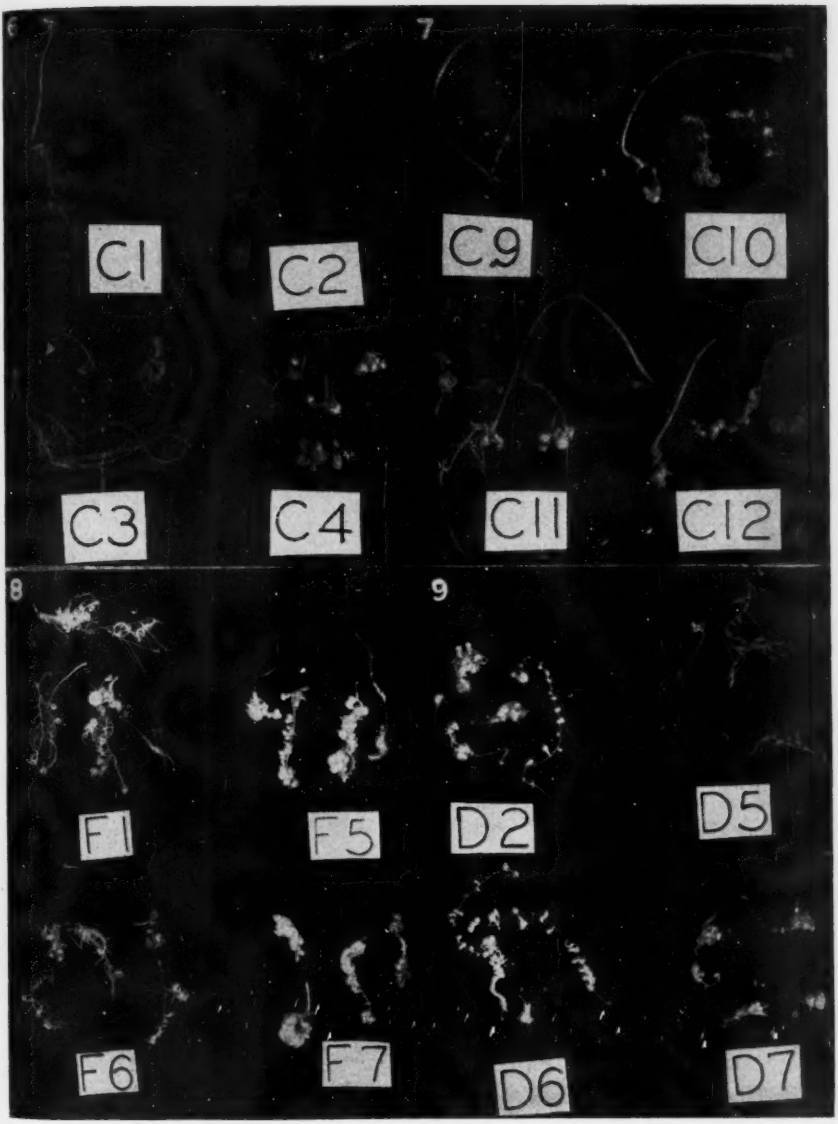
THE GROWTH SUBSTANCE.—This particular type of atypical growth of cabbage roots can be induced by all the chloro-substituted phenoxyacetic acids tested (p-chloro-, o-chloro-, 2,4-dichloro-, and 2,4,5-trichlorophenoxyacetic acids) and also by beta-naphthoxyacetic acid. Optimum concentration for the phenoxyacetic acids was found to be about $1 \times 10^{-6}$ *M*.

Other synthetic growth substances, including indoleacetic acid (IAA), naphthaleneacetic acid (NAA), and triiodobenzoic acid (TIBA) were tested in a range from $10^{-7}$ to $10^{-3}$ *M*. Maleic hydrazide was also tested at 0.002–0.2%. These growth substances did not induce a typical growth resembling that produced in the presence of POA, despite the fact that the highest concentrations used were in the region of physiological toxicity. At appropriate concentrations in the ranges tested all the growth substances did have physiological and morphological action on the seedlings. IAA and NAA inhibited primary-root elongation and induced excess laterals, thus acting similarly to POA in this respect. In distinction to the action of POA, however, no fusion of the laterals to produce the plates and wings was ever observed. These experiments are described in detail in a following paper (**19**).

THE YEAST-EXTRACT COFACTOR.—The enhancement by yeast extract of the tumor-forming ability of POA is illustrated in figure 6. The root systems pictured were grown attached to the entire seedling for 12 days on basal medium ($C_1$), or with supplements of POA ($C_3$), or yeast extract ($C_2$), or the two combined ($C_4$). It can readily be seen that the roots developed vigorous atypical growths only in the presence of the two combined factors.

Investigation of the yeast-extract effect described above eventually led to the discovery that many compounds containing reduced nitrogen could be substituted for yeast extract (table 1). Activity of these compounds was determined following 2 weeks' growth in the presence of the compound and an intermediate concentration of POA. At the end of the growth period the tumorous growth was observed and compared visually with control plants grown on POA alone.

Figure 7 shows the appearance of root systems of seedlings grown on media containing four different nitrogen sources. Although there was initiation of numerous root primordia in the presence of hydroxyproline ($C_9$), proliferation of




FIGS. 6–9.—Figs. 6–8, formation of fasciated outgrowths on roots of cabbage seedlings under influence of POA and different nitrogen sources. Fig. 6, $C_1$ plants were grown on basal medium, $C_2$ on basal medium plus yeast extract, $C_3$ on basal plus POA $8\times10^{-7}$ *M*, and $C_4$ on basal plus POA and yeast extract. Note formation of tumors only in presence of two factors combined. ×0.5. Fig. 7, seedling roots grown on basal medium plus POA $8\times10^{-7}$ *M* plus ($C_9$) hydroxyproline, ($C_{10}$) arginine, ($C_{11}$) ornithine, and ($C_{12}$) glycine. Note absence of proliferations with hydroxyproline as nitrogen source, compared with other amino acids. Fig. 8, seedling roots grown on ($F_1$) basal plus POA, and with supplements of ($F_5$) glycine, (F6) proline, and ($F_7$) ammonium chloride. High activity of ammonium chloride is indicated by solid appearance of root systems. Fig. 9, inhibition of tumor formation by high pH, and reversal of inhibition by addition of extra ammonium chloride. Root systems pictured above $D_2$ were induced to become tumorous by $8\times10^{-6}$ *M* POA plus $5\times10^{-4}$ *M* arginine; in the media for roots ($D_5$) the same components were present, but pH was maintained above 6.0 by succinate buffer at $8\times10^{-3}$ *M*. Reversal of this inhibition is seen for root systems shown above D6 and $D_7$, to which $3\times10^{-3}$ *M* and $8\times10^{-3}$ *M* ammonium chloride were added, respectively, in addition to medium used for roots in $D_5$.

lateral extensions was slight in comparison with that of seedlings receiving the same concentration of arginine (C10), ornithine (C11), or glycine (C12). Glycine (F5), proline (F6), and ammonium chloride (F7) are compared with the control of POA alone in figure 8. The extreme effectiveness of ammonium chloride can be seen in the complete lack of any normal appearing laterals with this nitrogen source, whereas a few occasionally appear when other nitrogen sources are used.

Since activity in supplementing the POA effect is not limited to any specific compounds but is shared by a number of nitrogen-containing chemicals, it appears likely that the function can be performed by any available nitrogen sources, such as amino or amide groups. The carbon skeleton to which the nitrogen is attached probably influences only the availability of the nitrogen and has no direct relationship to the function of the compound in augmentation of POA. This conclusion is supported by the high activity of ammonium chloride as well as by the much greater activity of asparagine and glutamine than of aspartic or glutamic acid.

The lack of activity of glutamate or aspartate was quite marked and was somewhat surprising, since these can be expected to supply amino groups quite readily through the transamination reactions known to occur in many plants (**22**). Lack of formation of tumors in the presence of these two compounds was found in subsequent experiments to be essentially an inhibition phenomenon related to the pH of the medium.

Lack of activity of nitrate or nitrite or of the aromatic amino acids and of purines and pyrimidines cannot be ascribed to any one particular cause at the present time. Poor uptake, lack of availability of the nitrogen atoms in a particular carbon structure, lack of relationship to specific pathways of utilization, and toxic effects are all possibilities to be considered. Nitrate was ineffective even at twenty times the concentration at which ammonium chloride showed a minimum effect, emphasizing the nonutilization of nitrogen in an oxidized state.

TABLE 1

ACTIVITY OF VARIOUS NITROGENOUS COMPOUNDS IN SUPPLEMENTING FORMATION OF FASCIATED CABBAGE-ROOT GROWTH BY POA. COMPOUNDS TESTED IN CONCENTRATIONS FROM $3\times10^{-4}$ TO $9\times10^{-4}$ *M*

| Effective | Moderately effective | Ineffective | |
|---|---|---|---|
| Yeast extract | Lysine | Tyrosine | Yeast nucleic acid |
| Casein hydrolyzate | Valine | Phenylalanine | Adenine |
| Peptone | Serine | Hydroxyproline | Guanine |
| | Threonine | Tryptophan | Uracil |
| Asparagine | Isoleucine | | Thymine |
| Glutamine* | Alpha-alanine | Leucine | |
| Ammonium chloride | Cysteine | Nor-leucine | Sodium nitrate† |
| | Homocysteine | Beta-alanine | Sodium nitrite |
| Urea | | Methionine | |
| Glycine | | Glutamic acid | Guanidine nitrate |
| Arginine | | Aspartic acid | Guanidine acetate |
| Ornithine | | | |
| Proline | | | |

* Filter sterilized. † Tested at concentrations up to $10^{-2}$ *M*.

pH SENSITIVITY.—Tumor formation under the influence of POA has been observed to be highly pH sensitive (fig. 9). Formation of the atypical growth was inhibited when the growth medium was buffered with organic acids, or with phosphate or carbonate buffers at pH 6.0 or above. At pH 5.5 or below tumor growth proceeded undisturbed. The inorganic salts of the growth medium by themselves were not strongly enough buffered to maintain a sufficiently high pH of the medium throughout the growth period. Effective concentrations of buffers for pH inhibition of tumor formation ranged upward from 0.003 *M*. Under this concentration, the pH of random aliquots of the medium remained high, but tumor formation was not affected.

The experiments on the effect of pH on the growth of tumors were complicated by the small volume and the static condition of the growth medium. These conditions led to the existence of pH drifts with time and gradients within the medium, which were only partially eliminated by the use of buffers. Such drifts and gradients would have reduced the significance of any precise correlation between pH and degree of tumor formation; hence no experiments along these lines were attempted.

## Discussion

Para-chlorophenoxyacetic acid is one of a series of halogen-substituted phenoxyacetic acid derivatives that have been brought into use as selective herbicides (**16, 17, 26, 36**). Typical growth-substance activity of these phenoxyacetic acid derivatives was first shown by ZIMMERMAN and HITCHCOCK (**52**) and has been substantiated by numerous other investigators. In view of these reports it would seem quite reasonable to consider the phenoxyacetic acid derivatives as additional compounds possessing or arousing auxin activity. However, in addition to their performance of the usual growth-substance functions, it has been claimed (**5, 6, 50, 51**) that these compounds, as well as beta-naphthoxyacetic acid, elicit a new set of plant physiological reactions. The reactions involved are modifications of leaves, flowers, roots, and stems in new and consistent ways, such that typical morphological patterns of the plants are altered. Because of the effect on plant morphology, such effects were called "formative" or "morphogenetic" effects. In order to avoid the implication of a unique control of morphological relationships by the phenoxyacetic and naphthoxyacetic compounds, such effects for the present will be called "POA-specific."

It seems evident that the present atypical growths can be classified with the other POA-specific reactions of plants. The characteristics of the tumor were not elicited by indoleacetic, naphthaleneacetic, or triiodobenzoic acid, while phenoxyacetic and naphthoxyacetic acids were functional. This specificity is identical with that previously reported for such POA-specific reactions. Further, a similar condition of nondivergent laterals has been noted previously to be one effect of the phenoxyacetic compounds on the aerial root of tropical grape (**52**), on pea roots (**28**), and on bean roots (**49**).

A complete description of the development of this fasciated growth is dependent on observation of the meristems during developmental stages and is lacking in the present instance, as in many investigations concerning the origins and development of fasciated structures recorded in the literature (see [**3**] for a discussion). It would seem likely

from the observations of separate vascular traces in longitudinal sections of the tumors (fig. 4*A*) that the cabbage tumors arise by nondivergence of meristems which arise collaterally.

The relationship of these atypical growths to naturally occurring fasciations (**2, 12, 46**) or to the initially nondivergent roots produced by the action of growth substances on stems (**7**) is not very clear and may be only a superficial one.

In any event, further growth of the meristems once initiated occurs for the most part in intimate connection and at equal rates; the net result is a fasciated plate of root tissue in which histological indication can be found of the number of roots involved in the growth. This fasciation can occur in two or possibly more directions at once, so as to produce deep plates that are laid down by rows of meristems two or more deep.

Vigorous and somewhat extended meristematic activity of the tumors is followed by a sudden expansion of cells to an enlarged, thin-walled state. The distance in which this cell enlargement phase occurs is much shorter than for normal roots. Vascular traces are differentiated, with scalariform tracheids, endodermis, and pericycle present. Epidermal cells are hypertrophied perpendicularly to the axis of growth, at least at first. Papillate cells present seem to bear little resemblance to typical root-hair cells.

The effect of 2,4-D on bean roots, as reported by WILDE (**49**), in many respects parallels the effects on cabbage-seedling roots. There were major differences, however; the most significant effect produced on bean roots was abnormal shoulders of nonvascularized tissue produced slightly in back of already established meristems. Such shoulders were present (although not investigated histologically) on cabbage roots but comprised only a small part of the atypical growth. Cabbage-root tissues continued to proliferate for a longer period of time and produced fasciated, vascularized plates along the entire length of primary roots exposed to POA; whereas the bean-root proliferation was relatively restricted. Anatomically, the tumorous plates of cabbage-root tissue under study are probably directly equivalent only to the stubby laterals found in a nondivergent condition at the distal ends of the bean-root shoulders.

The inhibition of tumor formation was previously observed and reported (**20**) to be a function of respiratory intermediates. The pH factor was not given full consideration at that time, and it has since become evident that the control of the pH of the medium is responsible for many of the inhibition phenomena previously observed. The inhibition by a high pH of POA activity in inducing cabbage-root tumors is in agreement with previous observations demonstrating lowered growth-substance activity at higher pH values (**1, 9, 11, 25, 31, 42, 43, 44**). Such results have been explained, for the most part, on the basis of the activity of only the undissociated growth-substance molecule. Exception to this point of view has been taken recently by LUNDEGARD (**23**) and RIETSEMA (**33**), who favor the view that indolacetic acid is active in the form of anions rather than as undissociated molecules. Any conclusion concerning the mechanism of the pH effect on tumor formation in the present instance is not warranted from the data obtained.

The yeast-extract cofactor effect has been definitely traced down to a reduced nitrogen effect, with ammonium chloride one of the most effective of the nitrog-

enous compounds tested. Although aromatic amino acids, purines, and pyrimidines, and nitrate or nitrite are not used to augment the POA effect, many other organic nitrogen sources are. The utilization of organic nitrogen sources by higher green plants or their parts in sterile or brief culture conditions has been by now fairly frequently recorded: thus radish leaf disks (10), etiolated pea parts (15), *Rumex* virus wound tumors in culture (30), orchid embryos (37), *Datura* embryos (35), sunflower crown-gall tumors in culture (34), and pea, clover, wheat, and barley plants (24, 32, 45) have all been shown to utilize one or another form of organic nitrogen. The evidence for utilization of urea by many plants is described in a recent review (27).

In the literature on utilization of organic nitrogen compounds, many if not most of the amino acids fed singly to plants proved to be toxic in varying degrees. These toxic effects are emphasized by the recent work of STEINBERG (39), in which it is shown that individual amino acids fed to tobacco plants in sterile culture can result in duplication of the symptoms of a physiological disease of tobacco in the field. Mixtures of amino acids are almost invariably less toxic than single compounds (29, 30). Toxic effects of ineffective amino acids cannot be ruled out in the present work.

Inactivity of nitrate nitrogen for augmentation of the POA effect recalls the findings of STAHLER and WHITEHEAD (38) that sugar-beet plants sprayed with 2,4-D accumulated excessively large amounts of nitrate. Such an observation might indicate a 2,4-D interference with nitrate reduction and its subsequent accumulation in at least some tissues. If this is a general effect of the phenoxyacetic compounds, it would explain the inability of nitrate to replace yeast extract; but such an explanation could not account for the inactivity of nitrite.

Interaction of synthetic auxins and organic nitrogen sources in the rooting and maintenance of cuttings has been studied in three cases. DOAK (14) found that naphthaleneacetic acid could stimulate the rooting of *Rhododendron* cuttings only when nitrogenous compounds were also present. Ammonium sulfate, arginine, and asparagine were quite effective in this capacity; adenine and guanine less so. In this instance both externally supplied auxin and extra nitrogen source were necessary for the physiological activity. Similarly for *Hibiscus* cuttings (41) rooting was dependent on auxin plus nitrogenous compounds and sugars (replacing the activity of leaves in the nutritive function). Active nitrogen sources in this case were ammonium sulfate, arginine, choline, betaine, and serine. A somewhat different situation is that reported for leafy cuttings of beans (40). Internal supplies of auxin and nitrogenous compounds complicated the results obtained in this case. However, external nitrogen added as potassium nitrate, adenine, asparagine, guanine, or choline (in that order) was effective in increasing the rooting of cuttings, for the most part in either the presence or absence of external auxin.

In the present experiments a number of nitrogenous compounds were found capable of augmenting an effect caused by a growth substance. The list, however, is not the same as those observed with cuttings. Whereas it seems possible to account for the action of nitrogen in the rooting of cuttings on a straight nutritional basis, such an explanation is not adequate in the present instance. This fact will be demonstrated in a subsequent publication. Also, the nitrogen augmen-

tation in the present instance applies to a physiological response that growth substances other than naphthoxy and phenoxy compounds do not seem to be able to elicit.

## Summary

1. The induction and appearance of atypical growths on the roots of cabbage seedlings under the influence of parachlorophenoxyacetic acid (POA) are described.

2. Synthetic growth substances inducing such growth are limited to the phenoxyacetic acid series and naphthoxyacetic acid.

3. A number of sources of reduced nitrogen augment the action of the growth substance in production of these tumors. Nonspecificity of the nitrogen sources is indicated by the effectiveness of a number of amino acids and amides and of ammonium chloride.

4. Formation of the atypical growths is inhibited by buffering the medium at pH 6.0 or above; whereas buffers at pH 5.5 or below have no inhibitory effect.

5. Histological preparations of typical roots and of the atypical growths show that the tumors consist essentially of fasciated plates of lateral root tissue, with growth occurring in a massive fashion.

6. Origin of the tumors is from the pericycle, or the pericycle and endodermis combined, of the primary root. Xylem, cortex, and epidermis are inactive in producing the growths and do not seem to be affected by POA.

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# STUDIES ON THE RELATION BETWEEN MOLECULAR STRUCTURE AND PHYSIOLOGICAL ACTIVITY OF PLANT GROWTH-REGULATORS. I. ABSCISSION-INDUCING ACTIVITY[1]

ROBERT L. WEINTRAUB, JAMES W. BROWN, JEAN C. NICKERSON, AND KATHARINE N. TAYLOR

## Introduction

During the last 20 years an ever increasing number of organic compounds has been shown to possess the property, in minute doses, of evoking pronounced developmental, physiological, and biochemical effects in plants. The term "plant growth-regulators" has come into fairly common usage to designate such chemicals. The structural diversity of the known growth-regulators has prompted several valuable investigations into the relation between molecular architecture and physiological potency, recently reviewed by NORMAN and WEINTRAUB (12). Insight into such a relationship would be expected to contribute to understanding of the mechanism of action of growth-regulators and might be of value in attempts to discover new and superior active compounds.

Inasmuch as plant growth-regulators induce a diversity of ultimate responses, some of which appear, at least superficially, to be quite unrelated, it must be borne in mind that the structural relationships which exist in a particular type of plant response may not hold for others. Furthermore, knowledge of the environmental and biological variables which are operative in influencing the plant responses is still so scanty that it may be unsafe to generalize broadly from results obtained by the use of a particular technique or single species. This paper is the first of a series dealing with the relation of structure to various types of activity.

Among the processes influenced by growth-regulators, that of abscission—the natural separation of leaves, floral parts, fruits, or foliage branches from the plant brought about by structural alterations in particular groups of cells—is of considerable interest both intrinsically and practically. Chemical techniques for retarding preharvest drop of orchard fruits are widely practiced, and attention has been given to methods of preventing early abscission of leaves, flowers, and berries from ornamental plants, wreaths, cut flowers, and the like. Premature, or induced, abscission is desired in other cases, such as blossom thinning of heavy-setting varieties of fruit trees and defoliation of cotton plants so as to facilitate picking.

Compounds belonging to the principal structural classes of growth-regulators (indolylaliphatic acids, α-naphthylaliphatic acids, and ring-substituted phenoxyaliphatic acids) generally exhibit a strong retarding effect upon abscission. On the other hand, 2,3,5-triiodobenzoic acid (TIBA) has been reported (6, 16) to promote abscission in certain plants. The present paper describes a further study of the action of TIBA in causing abscis-

[1] Portions of the data included in this paper were presented at the twenty-fourth annual meeting of the American Society of Plant Physiologists (New York, December 29, 1949) and at the Seventh International Botanical Congress (Stockholm, July 19, 1950).

sion in the garden bean, *Phaseolus vulgaris* var. Black Valentine, and of the comparative activities of a number of related compounds.

The abscission induced in beans is of a type common in immature and herbaceous stems, floral parts, and young fruits, in which no definite abscission zone is apparent, and the separation layer is formed very shortly before abscission (5). Separations of this type may occur at various positions in the internode and petioles; the precise location in chemically induced abscission appears to be related to the dosage of the applied abscissant. With doses not greatly exceeding the effective threshold, abscission was found predominantly in the internode subjacent to the treated leaf (figs. 1, 2) and much less frequently in the petiole or petiolules of this leaf. Considerably larger doses sometimes induced additional abscission layers in the stems or leaves above the treated leaf.

## Experimentation and observations

### Methods

The method of application of the growth-regulators was that described by Brown and Weintraub (3). Solutions of the compounds in 95% ethanol containing 1% Tween 20 were placed on the terminal buds of week-old seedlings, and the plants were cultured in the greenhouse until the first trifoliolate leaves of untreated controls were judged to have expanded to about two-thirds the normal maximal area. This required 6–10 days, depending upon the season. Each compound was tested a number of times at a series of dosages spanning the range between that producing no effect and that causing either complete abscission or complete suppression of growth. In each experiment sixteen plants were used to test each dosage level. At the end of the experimental culture period counts were made of the number of plants showing either complete abscission or an unmistakable separation layer above the primary leaf node.

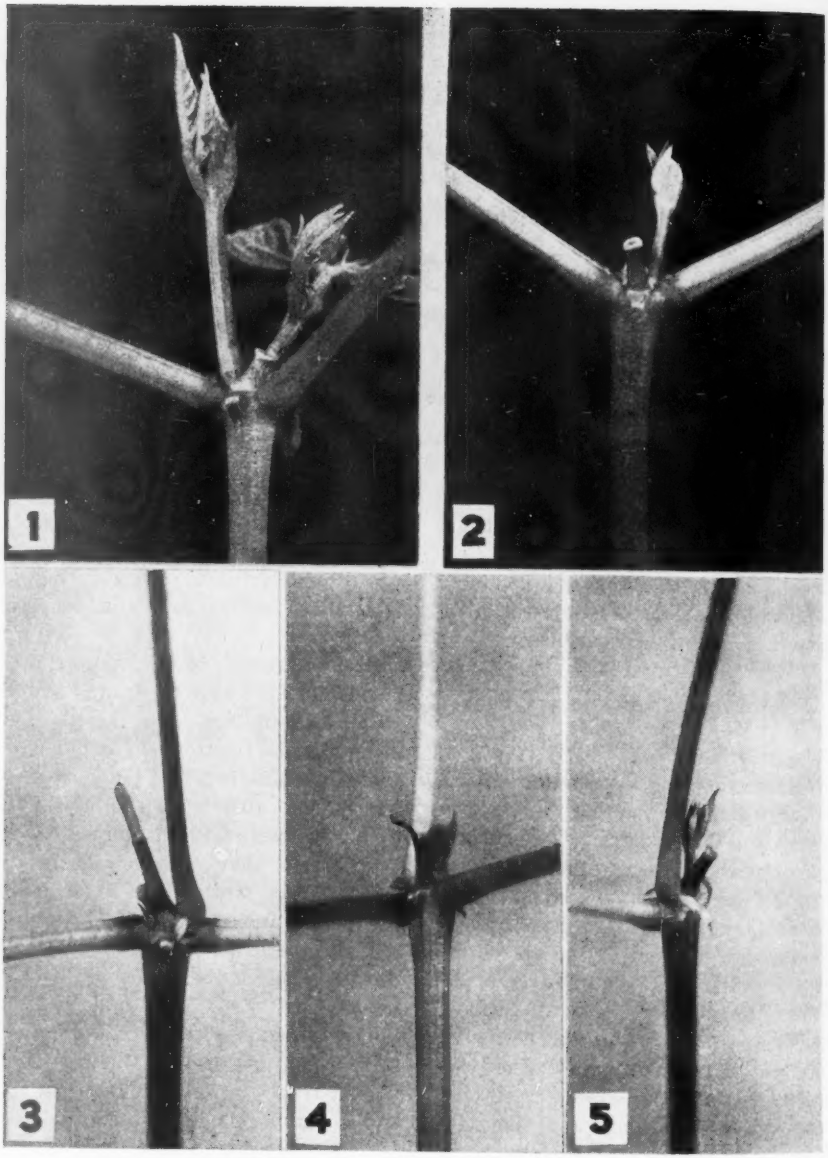
The responsiveness of the plants is markedly influenced by the stage of development at time of treatment, so that careful selection is required in order to achieve reproducible results. The sensitivity of each succeeding trifoliolate leaf appears to be maximal at the stage just prior to its unfolding from the bud.

### Influence of environment

During the course of the experiments, which extended over a 2-year period, it became apparent that there was a marked seasonal influence upon the sensitivity of the plants. Abscission was induced by lower doses during November, December, and January than at other seasons.

Previous studies of abscission reported in the literature have implicated temperature, air humidity, water supply, light intensity, and photoperiod as influential environmental factors. Tests of these factors indicated that the first three were sufficiently uniform throughout the year not to have been responsible for the observed variability. It is highly suggestive, furthermore, that the most sensitive period coincides with that of shortest photoperiod and lowest light intensity.

In experiments in which plants were transferred, just prior to treatment with TIBA, from the greenhouse to a controlled environment (illumination by Daylight fluorescent lamps; intensity approximately 900 foot-candles; photoperiod = 14 hours; temperature = 28 ± 1° C.; relative humidity = 50–70%), it was not possible to demonstrate any




FIGS. 1–5.—Successive stages in abscission of first internode of Black Valentine bean seedlings induced by application of 2,3,5-triiodobenzoic acid (TIBA) to terminal bud (figs. 1, 2) or by excision of terminal bud (figs. 3, 4, 5).

clear-cut effect of either photoperiod or intensity. It was found, however, that sensitivity was greatly increased by culturing the plants from emergence in the controlled room rather than in the greenhouse; under such conditions the sensitivity was somewhat greater than that in the greenhouse during the winter months, and highly reproducible results were obtained in successive tests.

room facilities, these tests were performed in the greenhouse.

2,3,5-TRIHALOGENATED BENZOIC ACIDS.—The twenty-seven possible 2,3,5-trisubstituted benzoic acids containing iodine, bromine, and chlorine were synthesized at Camp Detrick by H. E. THOMPSON, J. F. OWINGS, and W. M. NUTTER using procedures designed to yield only the desired isomers. Inasmuch as several of these compounds do not appear to have been previously described, the melting points and neutralization equivalents of this series are recorded in table 1. Preparative methods and other data for the new compounds are to be published elsewhere.

TABLE 1

PROPERTIES OF 2,3,5-TRIHALOGENATED BENZOIC ACIDS

| BENZOIC ACID | MELTING POINT (° C.) | | MOLECULAR WEIGHT | | NEUTRALIZATION EQUIVALENT FOUND |
|---|---|---|---|---|---|
| | Observed* | Literature | Calculated | Found† | |
| 2-Bromo-3-chloro-5-iodo- | 224–225 | | 361.3 | 371.3 | 354.3 |
| 2-Bromo-5-chloro-3-iodo- | 154–155 | | 361.3 | 368.2 | 364.2 |
| 3-Bromo-2-chloro-5-iodo- | 194–195 | | 361.3 | 370.0 | 361.0 |
| 3-Bromo-5-chloro-2-iodo- | 158–159 | | 361.3 | 371.4 | 366.1 |
| 5-Bromo-2-chloro-3-iodo- | 135–136 | | 361.3 | 352.1 | 361.0 |
| 5-Bromo-3-chloro-2-iodo- | 165–166 | | 361.3 | 346.5 | 361.2 |
| 2-Bromo-3,5-dichloro- | 178–179 | 178 | 269.9 | 264.7 | 267.0 |
| 3-Bromo-2,5-dichloro- | 185–186 | | 269.9 | 259.1 | 264.0 |
| 5-Bromo-2,3-dichloro- | 183–186 | | 269.9 | 274.8 | 270.0 |
| 2-Chloro-3,5-dibromo- | 186–187 | 180–181 | 314.3 | 309.1 | 314.1 |
| 3-Chloro-2,5-dibromo- | 197–198 | | 314.3 | 319.5 | 311.5 |
| 5-Chloro-2,3-dibromo- | 154–155 | | 314.3 | 299.9 | 309.0 |
| 2-Bromo-3,5-diiodo- | 209–210 | | 452.8 | 445.6 | 448.0 |
| 3-Bromo-2,5-diiodo- | 197–198 | | 452.8 | 460.0 | 449.1 |
| 5-Bromo-2,3-diiodo- | 170–171 | | 452.8 | 443.3 | 450.7 |
| 2-Chloro-3,5-diiodo- | 207–208 | 206–207 | 408.3 | 403.2 | 406.6 |
| 3-Chloro-2,5-diiodo- | 209–212 | | 408.3 | 421.0 | 399.8 |
| 5-Chloro-2,3-diiodo- | 168–170 | | 408.3 | 398.4 | 406.1 |
| 2,3-Dibromo-5-iodo- | 202–203 | | 405.8 | 399.0 | 404.0 |
| 2,5-Dibromo-3-iodo- | 138–140 | | 405.8 | 417.2 | 405.2 |
| 3,5-Dibromo-2-iodo- | 184–185 | | 405.8 | 393.6 | 406.0 |
| 2,3-Dichloro-5-iodo- | 207–213 | | 316.9 | 324.2 | 316.7 |
| 2,5-Dichloro-3-iodo- | 149–150 | | 316.9 | 311.1 | 315.0 |
| 3,5-Dichloro-2-iodo- | 171–172 | | 316.9 | 301.0 | 312.0 |
| 2,3,5-Tribromo- | 185–186 | 193–194 | 358.8 | 352.0 | 358.0 |
| 2,3,5-Trichloro- | 171–172 | 163 | 225.4 | 225.6 | 221.0 |
| 2,3,5-Triiodo- | 226–230 | 224–226 | 499.8 | 497.5 | 491.5 |

* Fisher-Johns apparatus. † Ebulliscopic in benzene.

## STRUCTURE AND ABSCISSION-INDUCING ACTIVITY

In attempting to ascertain the structural requirements for the abscission response a large number of compounds were tested. Owing to limited controlled-

In order to obtain results as comparable as possible from experiment to experiment, all these substances were retested during the November–January period of highest sensitivity. 2,3,5-Triiodobenzoic acid was included as a standard compound in each test as a check on the consistency of response.

The percentage of the population showing abscission was directly proportional to the dose applied, as exemplified in figure 6. The available evidence indicates that the *relative* activities of the various compounds remain constant regardless of environmental conditions, though the *absolute* doses required for a chosen level of response may vary somewhat with the season. It has been thought desirable, nevertheless, to express the results in absolute terms; the

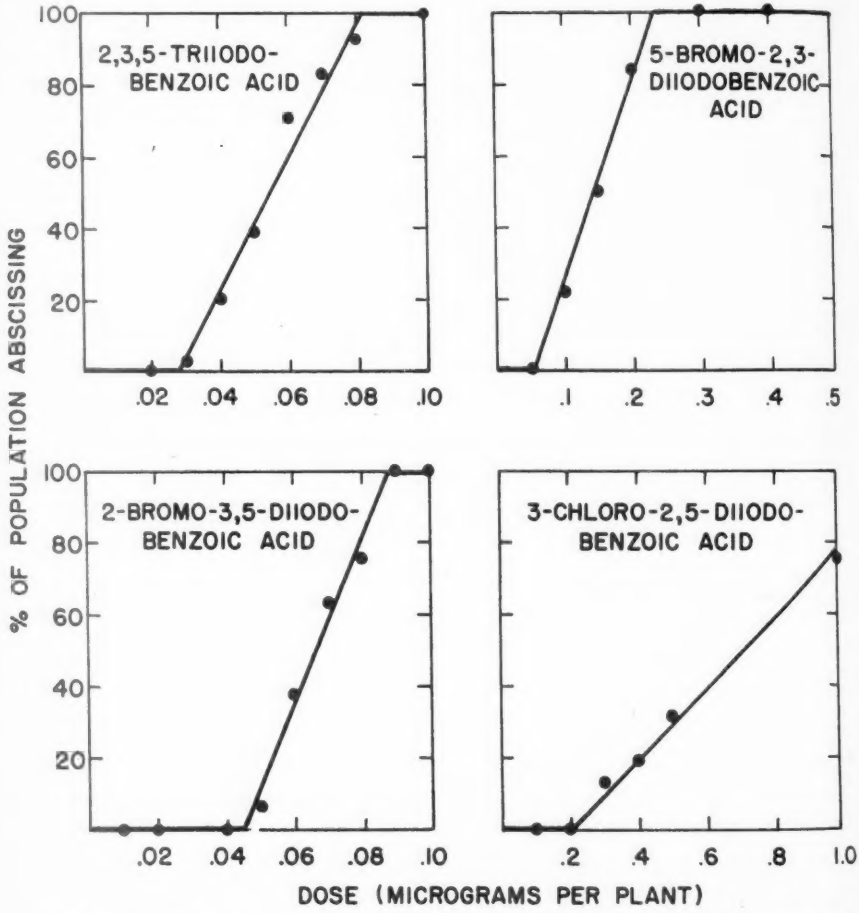



FIG. 6.—Relationship between abscission response and applied dose of representative 2,3,5-trihalogenated benzoic acids.

relative activities may be readily calculated therefrom. Since the slopes of the dosage-response curves differed considerably among the several compounds, graphically determined threshold values (the intercepts on the dose co-ordinate) were used as a basis for the comparison of activities.

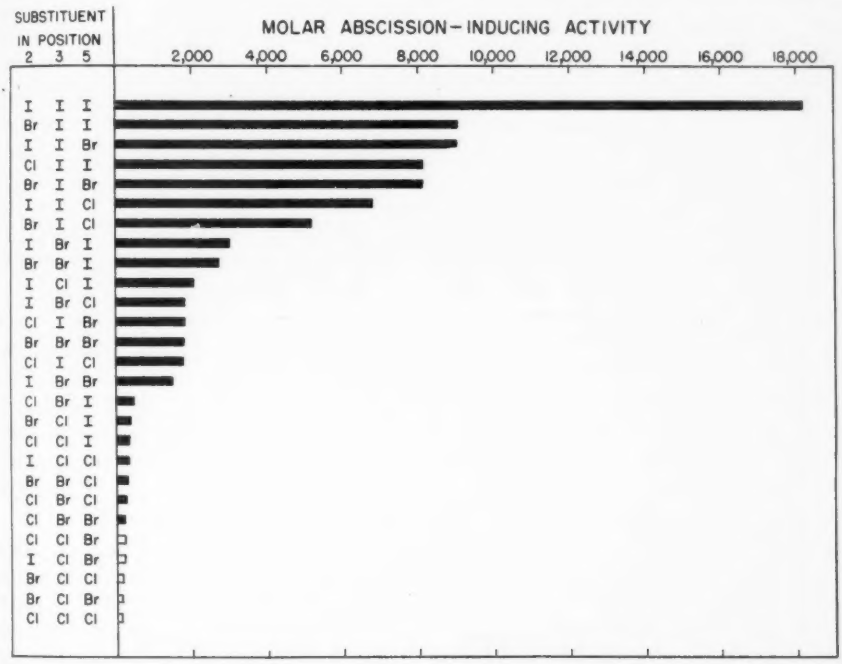



FIG. 7.—Abscission-inducing activity of 2,3,5-trihalogenated benzoic acids (= molecular weight divided by threshold dose in micrograms per plant). Unshaded bars signify that activity, which could not be precisely determined, is no greater and probably less than that indicated.

The results for this series of compounds are presented in figure 7. To compare activities on a molecular basis the reciprocals of the threshold values in micrograms per plant have been multiplied by the respective molecular weights.

It is apparent that activity is influenced not only by the particular halogen atoms present in the molecule but also by the specific position which each occupies. The activating influence of the halogens is clearly in the order I > Br > Cl. The relative importance of each position is more difficult to assess inasmuch as the effect of a particular atom in one position seems to be modified by the nature of the atoms in the other positions. Comparisons between the various pairs of compounds indicate that the substituent occupying position 3 is of greater significance than are those in positions 2 and 5.

OTHER BENZOIC ACIDS.—Exploratory tests were carried out with approximately ninety mono-, di-, and trisubstituted benzoic acids in addition to the above series. In these tests, which were performed throughout the year, rather

widely spaced doses were employed, and only the order of magnitude of the activity was determined. Of the compounds investigated, only 2-bromo-5-iodobenzoic acid and 2,5-dibromobenzoic acid, with molar activities of approximately 4000 and 1400, respectively, were as active as the 2,3,5-trihalogenated acids. 2,5-Diiodobenzoic, 3,4,5-triiodobenzoic, and 2-hydroxy-5-nitrobenzoic acids exhibited somewhat lower activity, while the remaining compounds (included in table 2) were still less effective by at least an order of magnitude.

MISCELLANEOUS COMPOUNDS.—Abscission-inducing activity is not restricted to the substituted benzoic acids. In preliminary tests with approximately five hundred other compounds, representing a variety of structures, about one-tenth showed some activity when applied at sufficiently high doses. The most effective of these, which were of the same order of activity as the least potent 2,3,5-trihalogenated benzoic acids, were the following:

5,7-Dibromo-8-quinolyloxyacetic acid
2,4-Dinitrophenylacetic acid
Dithiobiuret
3,6-Endoxohexahydrophthalic acid
Iodoacetic acid
Isopropyl N-(3-bromophenyl)-carbamate
Isopropyl N-(3-chlorophenyl)-carbamate
Maleic hydrazide
N-1-Naphthylphthalamic acid
Phenylmercuric acetate

The other compounds which exhibited some activity are listed in table 2, while those found to be inactive at the doses tested are included in table 3. It is to be noted that although most of the latter were tested at dosages up to 10 or 20 μgm. per plant, some of them could be assayed only at considerably lower levels owing to insolubility or, particularly in the case of certain phenoxy derivatives, to high formative activity.

## MECHANISM OF ABSCISSION

The sequence of events leading to abscission in herbaceous organs is very incompletely understood. Cells in all the tissues undergo a series of transverse divisions leading to formation of a layer several cells in width across the organ. Separation of these cells appears to result from chemical changes involving pectic substances of the middle lamellae and perhaps also of the cell walls (10, 13). Experiments with *Coleus* indicate that under normal conditions the cell divisions are inhibited by an auxin produced in the leaf blade. Abscission was found to occur rather quickly if the lamina was excised but could be greatly retarded by application of 3-indoleacetic acid (IAA) or related substances (7, 8, 9, 11).

The situation seems to be very much the same in the young bean stem, for decapitation of the terminal bud results in abscission of the subjacent internode (figs. 3–5). As in chemically induced abscission, the degree of responsiveness depends upon the developmental stage of the bud at time of treatment.

It might be expected, therefore, that any condition which tends to restrict the production, or to promote the destruction, of auxin in the leaf would favor the onset of abscission. Quite possibly the greater sensitivity of plants cultured under relatively low light intensities may be due to a relatively low auxin content.

## ANTAGONISM OF TIBA BY AUXINS

TIBA appears to act by interfering with the auxin mechanism, since its abscission-inducing activity can be counteracted by concurrent application of auxins. The results of a typical experiment with IAA (table 4) show that abscission was prevented completely by 6–9 moles IAA per mole TIBA. β-3-Indolepropionic acid and γ-3-indolebutyric acid

TABLE 2

COMPOUNDS HAVING RELATIVELY LOW ABSCISSION-INDUCING ACTIVITY

2-Allyl-4-chlorophenoxyacetic acid
Allyldiphenylacetic acid
9-Allylfluorene-9-carboxylic acid
2-Amino-5-iodobenzoic acid
Benzimidazole
2-Benzoylbenzoic acid
2-Benzoyl-4-bromobenzoic acid
5-Bromo-2-hydroxybenzoic acid
2-Bromo-3-nitrobenzoic acid
2-Bromo-4-nitrobenzoic acid
2-Bromo-5-nitrobenzoic acid
3-Bromo-2-nitrobenzoic acid
3-Bromo-4-nitrobenzoic acid
3-Bromo-5-nitrobenzoic acid
3-Bromo-6-nitrobenzoic acid
4-Bromo-2-nitrobenzoic acid
4-Bromo-3-nitrobenzoic acid
2-(*p-tert*-Butylphenoxy-ethanol
β-Carboxyethylbenzothiazyl-2-sulfide
4-Chloro-2-(β,γ-dichloropropyl)-phenoxyacetic acid
2-Chloro-4-methylphenoxyacetic acid
2-Chloro-3-nitrobenzoic acid
α-(4-Chlorophenoxy)-heptylic acid
3-(4′-Chlorophenoxy)-1-propanol
β-(4-Chlorophenoxy)-propionic acid
Coumarilic acid
2-Coumaronecarboxylic acid
3,5-Dibromoaniline
2,4-Dibromo-6-methylphenoxyacetic acid
2,4-Dichlorobenzoic acid
2,5-Dichlorobenzoic acid
3,4-Dichlorobenzoic acid
2,4-Dichloro-6-methylphenoxyacetic acid
α-(2,4-Dichlorophenoxy)-heptylic acid
1-(2′,4′-Dichlorophenoxy)-2-propanol
Di-(*p*-chlorophenyl)-trichloromethylmethane
Diethyl 2,4-dichloro-α-hydroxy-α-toluenephosphonate
2,6-Dihydroxybenzoic acid
3,5-Diiodobenzoic acid
3,5-Diiodo-2-hydroxybenzoic acid
3,5-Diiodo-4-hydroxybenzoic acid
Dimethylhydantoin
2,5-Dimethylphenol
3,4-Dimethylphenoxyacetic acid
3,5-Dimethylphenoxyacetic acid
2,5-Dinitrobenzoic acid
3,5-Dinotrobenzoic acid
3,5-Dinitro-2-fluorobenzoic acid
3,5-Dinitrosalicylic acid
Ethyl 4-chlorophenylcarbonate
Ethyl 2,4-dibromophenylcarbonate
4-Fluorobenzoic acid
4-Fluoro-3-nitrobenzoic acid
2-Hydroxy-5-iodo-3-methylbenzoic acid
2-Hydroxy-3-methylbenzoic acid
1-Hydroxy-2-naphthoic acid
3-Hydroxy-2-naphthoic acid
4-Hydroxy-3-nitrobenzoic acid
2-Iodo-3-nitrobenzoic acid
2-Iodophenoxyacetic acid
5-Iodosalicylic acid
2-Methoxy-4-methylphenoxyacetic acid
3-Methoxyphenoxyacetic acid
4-Methoxyphenoxyacetic acid
2-Methyl-3-nitrobenzoic acid
1-Naphthaleneacetic acid
1-Naphthoic acid
2-Naphthoic acid
2-Naphthoxyacetic acid
2-Nitrothiophene
α-(Phenoxy)-isobutyric acid
2-Pyridinecarboxylic acid hydrochloride
8-Quinolinoxyacetic acid
Sodium isopropylxanthate
*p*-Toluic acid
3,4,5-Tribromobenzoic acid
3,4,5-Trichlorobenzoic acid
3-(2′,4′,5′-Trichlorophenoxy)-1-propene
2,4,6-Trihydroxybenzoic acid
2,3,5-Triiodobenzamide
2,4,6-Triiodophenoxyacetic acid
α-(3,4,5-Trimethylphenoxy)-*n*-butyric acid
1,3,5-Trinitrobenzene
2,4,6-Trinitrobenzoic acid
2,4,6-Trinitrophenol

TABLE 3

COMPOUNDS NOT INDUCING ABSCISSION

3-(Acetamido)-phenoxyacetic acid
2-Acetyl-4-chlorophenoxyacetic acid
Acetyl peroxide
4-Acetylphenoxyacetic acid
Acrolein-sodium bisulfite compound
Adenine sulfate
Allyl 4-chlorophenoxyacetate
Allyl 2,4-dichlorophenoxyacetate
4-Aminoazobenzene
3-Aminobenzoic acid
4-Aminobenzoic acid
5-Amino-2,4-dichlorophenoxyacetic acid
2-Amino-3,5-diiodobenzoic acid
β-Aminoethyl bis-4-chlorophenoxyacetate
1-Amino-2-naphthol-4-sulfonic acid
4-Aminophenol
2-Aminophenoxyacetic acid
3-Aminophenoxyacetic acid
4-Aminophenoxyacetic acid
4-Aminophenylacetic acid
5-Aminosalicylic acid
2-Amyl-4-chlorophenoxyacetic acid
*n*-Amyl 2,4-dichlorophenoxyacetate
Aniline hydrochloride
Anthranilic acid
Barbituric acid
Benzoic acid
Benzoic anhydride
Benzoyl peroxide
4-Benzoylphenoxyacetic acid
2-Benzyl-4-chlorophenoxyacetic acid
N,N′-Bis-(3-chlorophenyl)-urea
N,N′-Bis-(2,4-dichlorophenyl)-urea
Boric acid
2-Bromoanisole
4-Bromoanisole
2-Bromobenzoic acid
3-Bromobenzoic acid
4-Bromobenzoic acid
2-Bromo-4-*tert*-butylphenoxyacetic acid
4-Bromo-2-carboxy-6-methylphenoxyacetic acid
2-Bromo-4-chlorophenoxyacetic acid
4-Bromo-2-chlorophenoxyacetic acid
5-Bromo-2,4-dichlorophenoxyacetic acid
2-Bromo-3,5-dinitrobenzoic acid
β-Bromoethyl 2,4-dichlorophenoxyacetate
4-Bromo-2-formylbenzoic acid
3-Bromo-2-hydroxy-5-sulfobenzoic acid
2-Bromo-5-iodobenzoic acid
5-Bromo-2-iodobenzoic acid
2-Bromo-4-methylphenoxyacetic acid
3-Bromo-5-methylphenoxyacetic acid
4-Bromo-2-methylphenoxyacetic acid
*o*-Bromonitrobenzene
4-Bromo-2-oximinoethylphenoxyacetic acid
2-Bromophenoxyacetic acid
3-Bromophenoxyacetic acid
4-Bromophenoxyacetic acid
2-(4′-Bromophenoxy)-ethanol
3-(4′-Bromophenoxy)-1-propene
4-Bromophenylacetic acid
N-2-Bromophenyl-N′-2-chlorophenylurea
N-4-Bromophenyl-N′-2-chlorophenylurea
N-4-Bromophenyl-N′-3-chlorophenylurea
2-Bromo-4-phenylphenoxyacetic acid
N-4-Bromophenyl-N′-phenylurea
N-(4-Bromophenyl)-urea
N,N′-Bis-(4-bromophenyl)-urea
β-(Butoxy)-ethyl 2,4-dichlorophenoxyacetate
*n*-Butyl 4-chlorophenoxyacetate
2-*sec*-Butyl-4-chlorophenoxyacetic acid
N-*sec*-Butyl cinnamide
*n*-Butyl 2,4-dichlorophenoxyacetate
*sec*-Butyl 2,4-dichlorophenoxyacetate
*tert*-Butyl hydroperoxide
4-*tert*-Butylphenoxyacetic acid
2-(4′-*tert*-Butylphenoxy)-ethyl acetate
α-(4-*tert*-Butylphenoxy)-propionic acid
*n*-Butyl 2,4,5-trichlorophenoxyacetate
2-Carbomethyl-4-chlorophenoxyacetic acid
2-Carboxy-4-chloro-6-methylphenoxyacetic acid
2-Carboxy-4-chlorophenoxyacetic acid
6-Carboxy-2,4-dichlorophenoxyacetic acid
2-Carboxy-6-methylphenoxyacetic acid
2-Carboxy-1-naphthoxyacetic acid
2-Carboxyphenoxyacetic acid
3-Carboxyphenoxyacetic acid
4-Carboxyphenoxyacetic acid
4-Chloroanisole
2-Chlorobenzoic acid
3-Chlorobenzoic acid
4-Chlorobenzoic acid
2-Chloro-4-*tert*-butylphenoxyacetic acid
2-Chlorocinnamic acid
4-Chlorocinnamic acid
6-Chlorocoumarin
4-Chloro-2-cyanomethylphenoxyacetic acid
4-Chloro-2-cyclohexylphenoxyacetic acid
2-Chloro-1,4-dihydroxybenzene
4-Chloro-3,5-dimethylphenoxyacetic acid
β-Chloroethyl 4-chlorophenoxyacetate
β-Chloroethyl 2,4-dichlorophenoxyacetate
4-Chloro-2-ethylphenoxyacetic acid
Chlorohydroquinone
Chlorohydroquinone-O,O-diacetic acid

TABLE 3—*Continued*

2-Chloro-3-hydroxyjuglone
2-Chloro-5-iodobenzoic acid
2-Chloro-4-iodophenoxyacetic acid
4-Chloro-2-iodophenoxyacetic acid
Chloromaleic anhydride
Chloromaleic hydrazide
4-Chloromandelic acid
2-Chloromethyl-4-chlorophenoxyacetic acid
4-Chloro-2-methylphenoxyacetic acid
4-Chloro-3-methylphenoxyacetic acid
6-Chloro-2-methylphenoxyacetic acid
α-(2-Chloro-4-methylphenoxy)-propionic acid
7-Chloro-1-naphthoxyacetic acid
4-Chloro-1-naphthylacetohydrazide
2-Chloro-4-nitrophenoxyacetic acid
4-Chloro-2-nitrophenoxyacetic acid
4-Chlorophenetole
4-Chlorophenoxyacetamide
4-Chlorophenoxyacetanilide
4-Chlorophenoxyacet-(β-hydroxyethyl)-amide
2-Chlorophenoxyacetic acid
3-Chlorophenoxyacetic acid
4-Chlorophenoxyacetic acid
4-Chlorophenoxyacetic acid, S-(1-naphthylmethyl)-isothiourea salt
4-Chlorophenoxyacetic phenylhydrazide
4-Chlorophenoxyaceto-*p*-anisidide
4-Chlorophenoxyaceto-4′-bromoanilide
4-Chlorophenoxyaceto-2′-chloroanilide
4-Chlorophenoxyaceto-3′-chloroanilide
4-Chlorophenoxyaceto-2′,5′-dichloroanilide
4-Chlorophenoxyaceto-di-(β-hydroxyethyl)-amide
4-Chlorophenoxyaceto-2′,4′-dimethylanilide
4-Chlorophenoxyaceto-4′-iodoanilide
4-Chlorophenoxyaceto-α-naphthalide
4-Chlorophenoxyaceto-β-naphthalide
4-Chlorophenoxyacetone
4-Chlorophenoxyacetonitrile
4-Chlorophenoxyaceto-2′-nitroanilide
4-Chlorophenoxyaceto-3′-nitroanilide
4-Chlorophenoxyaceto-4′-toluidide
4-Chlorophenoxyaceto-2′,4′,6′-trichloroanilide
4-Chlorophenoxyaceto-3′-(trifluoromethyl)-anilide
1-(*p*-Chlorophenoxyacetylamino)-8-hydroxynaphthalene-3,6-disulfonic acid
1-(*p*-Chlorophenoxyacetylamino)-naphthalene-4-sulfonic acid
1-(*p*-Chlorophenoxyacetylamino)-naphthalene-8-sulfonic acid
4-Chlorophenoxyacetyl chloride
α-(2-Chlorophenoxy)-*n*-butyric acid
α-(3-Chlorophenoxy)-*n*-butyric acid
α-(4-Chlorophenoxy)-*n*-butyric acid
γ-(4-Chlorophenoxy)-*n*-butyric acid
N-(*p*-Chlorophenoxyacetyl)-tri-(hydroxymethyl)-methylamine
4-Chlorophenoxy-α-(4′-chlorophenyl)-acetic acid
2-(4′-Chlorophenoxy)-ethanol
4-Chlorophenoxyfumaric acid
1-(2′-Chlorophenoxy)-2-propanol
3-(4′-Chlorophenoxy)-propene
α-(2-Chlorophenoxy)-propionic acid
α-(3-Chlorophenoxy)-propionic acid
α-(4-Chlorophenoxy)-propionic acid
β-(2-Chlorophenoxy)-propionic acid
4-Chlorophenylacetic acid
4-Chlorophenylallyl carbonate
4-Chlorophenyl 4′-chlorophenoxyacetate
N-2-Chlorophenyl-N′-4-chlorophenyl-urea
N-4-Chlorophenyl-N′-3-chlorophenyl-urea
N-(3-Chlorophenyl)-N′,N′-(pentamethylene)-urea
2-(2′-Chlorophenyl)-phenoxyacetic acid
2-(4′-Chlorophenyl)-phenoxyacetic acid
N-4-Chlorophenyl-N′-phenylurea
N-2-Chlorophenylphthalamic acid
N-(4-Chlorophenyl)-urea
N,N′-Bis-(4-chlorophenyl)-urea
2-(3′-Chlorophenylureido)-3,5-diiodobenzoic acid
N-2-Chlorophenyl-N′-2-xenylurea
N-3-Chlorophenyl-N′-2-xenylurea
4-Chloro-2-propylphenoxyacetic acid
Cinnamic acid
Cumene hydroperoxide
Cyclohexanecarboxylic acid
Cyclohexanone peroxide
4-Cymene hydroperoxide
2,5-Dibromobenzoic acid
2,6-Dibromobenzoquinone-4-chloroimide
2,6-Dibromo-3-hydroxyjuglone
2,4-Dibromophenoxyacetic acid
3,5-Dibromophenoxyacetic acid
3-(2′,4′-Dibromophenoxy)-1-propene
N-(2,4-Dibromophenyl)-urea
N,N′-Bis-(2,4-dibromophenyl)-urea
3,5-Dibromo-L-tyrosine
2,4-Di-*tert*-butyl-5-methylphenoxyacetic acid
Di-*tert*-butyl peroxide
N,N-Dicarboxyethylbenzenesulfonamide
Dichloralurea
2,5-Dichloroaniline
2,5-Dichloroaniline-4-sulfonic acid
2,4-Dichloroanisole
1,1-Dichloro-2,2-bis-(4′-chlorophenyl)-ethane
2,4-Dichlorocinnamic acid
2,4-Dichloro-3,5-dimethylphenoxyacetic acid

TABLE 3—*Continued*

α-(2,4-Dichloro-3,5-dimethylphenoxy)-propionic acid
2,6-Dichloro-4-methylphenoxyacetic acid
2,4-Dichloro-5-nitrophenoxyacetic acid
2,6-Dichloro-3-nitrophenoxyacetic acid
2,4-Dichlorophenetole
2,4-Dichlorophenol
2,4-Dichlorophenoxyacetaldehyde diethylacetal
2,4-Dichlorophenoxyacetamide
2,5-Dichlorophenoxyacetamide
α,α-Di-(4-chlorophenoxy)-acetamide
2,4-Dichlorophenoxyacetanilide
2,4-Dichlorophenoxyacet-(diethanol)-amide
2,4-Dichlorophenoxyacet-(diethyl)-amide
Di-(2-chlorophenoxy)-acetic acid
Di-(4-chlorophenoxy)-acetic acid
2,3-Dichlorophenoxyacetic acid
2,4-Dichlorophenoxyacetic acid
2,5-Dichlorophenoxyacetic acid
2,6-Dichlorophenoxyacetic acid
3,4-Dichlorophenoxyacetic acid
3,5-Dichlorophenoxyacetic acid
2,4-Dichlorophenoxyacetic anhydride
2,4-Dichlorophenoxyacetic hydrazide
2,4-Dichlorophenoxyacetic acid hydrazone of levulinic acid
2,4-Dichlorophenoxyacet-(methyl)-amide
2,4-Dichlorophenoxyaceto-*p*-anisidide
2,4-Dichlorophenoxyaceto-4′-bromanilide
2,4-Dichlorophenoxyaceto-4′-carboxyanilide
2,4-Dichlorophenoxyaceto-4′-chloroanilide
2,4-Dichlorophenoxyaceto-2′,5′-dichloroanilide
2,4-Dichlorophenoxyaceto-2′,4′-dimethylanilide
2,4-Dichlorophenoxyacetoguanidide
2,4-Dichlorophenoxyacetohydroxamic acid
2,4-Dichlorophenoxyaceto-α-naphthalide
2,4-Dichlorophenoxyaceto-β-naphthalide
2,4-Dichlorophenoxyaceto-2′-nitroanilide
2,4-Dichlorophenoxyacetopiperidide
2,4-Dichlorophenoxyaceto-4′-sulfonanilide
2,4-Dichlorophenoxyaceto-*p*-toluidide
2,4-Dichlorophenoxyaceto-2′,4′,6′-trichloroanilide
2,4-Dichlorophenoxyacetoxypropionic acid
2,4-Dichlorophenoxyaceturic acid
2,4-Dichlorophenoxyacetyl chloride
N-2,4-Dichlorophenoxyacetylurea
α-(2,4-Dichlorophenoxy)-*n*-butyric acid
γ-(2,4-Dichlorophenoxy)-*n*-butyric acid
ε-(2,4-Dichlorophenoxy)-caproic acid
ζ-(2,4-Dichlorophenoxy)-enanthic acid
1,2-Di-(4′-chlorophenoxy)-ethane
2-(2′,4′-Dichlorophenoxy)-ethanol
2-(2′,4′-Dichlorophenoxy)-ethylamine
Di-[2-(4-chlorophenoxy)-ethyl]-ether
N-[β-(2,4-Dichlorophenoxy)-ethyl]-phthalamic acid
2,4-Dichlorophenoxyformamide
2,4-Dichlorophenoxyfumaric acid
3-(2′,4′-Dichlorophenoxy)-1-propanol
3-(2′,4′-Dichlorophenoxy)-propene
α-(2,4-Dichlorophenoxy)-propionic acid
α-(2,5-Dichlorophenoxy)-propionic acid
α-(2,6-Dichlorophenoxy)-propionic acid
β-(2,4-Dichlorophenoxy)-propionic acid
2,4-Dichlorophenoxystearic acid
N-(2,4-Dichlorophenyl)-glycine
2,5-Dichlorophenylhydrazine
2,5-Dichlorophenylhydrazine-4-sulfonic acid
S-(2,4-Dichlorophenyl)-thioglycolic acid
N-2,4-Dichlorophenyl-N′-phenylurea
N-(2,4-Dichlorophenyl)-urea
2,6-Dichloroquinone
4-(Diethylamino)-benzaldoxime hydrochloride
Diethyl 4-chloro-α-hydroxy-α-toluenephosphonate
Diethyl 3,4-dichloro-α-hydroxy-α-toluenephosphonate
Diethyleneglycol dicarbamate
Diethyloxalate
Diethyl phenoxyethylmalonate
2,4-Difluorophenoxyacetic acid
2,4-Dihydroxybenzoic acid
2,5-Dihydroxybenzoic acid
3,4-Dihydroxybenzoic acid
5,7-Dihydroxydimethylcoumarin
3,4-Diiodobenzoic acid
2,5-Diiodobenzoic acid
2,4-Diiodophenoxyacetic acid
3,5-Diiodo-L-tyrosine
Diisopropylbenzene monohydroperoxide
2,3-Dimethylbenzoic acid
3,5-Dimethylphenoxyacetamide
2,3-Dimethylphenoxyacetic acid
2,4-Dimethylphenoxyacetic acid
2,5-Dimethylphenoxyacetic acid
α-(3,4-Dimethylphenoxy)-*n*-butyric acid
α-(3,5-Dimethylphenoxy)-*n*-butyric acid
α-(2,4-Dimethylphenoxy)-propionic acid
α-(2,5-Dimethylphenoxy)-propionic acid
β-(3,5-Dimethylpheonxy)-propionic acid
3,5-Dimethylpyrazole
3,4-Dinitrobenzoic acid
2,4-Dinitro-6-methylphenol
2,4-Dinitro-1-naphthol-7-sulfonic acid
2,4-Dinitrophenol
2,4-Dinitrophenoxyacetic acid
Diphenylacetic acid

TABLE 3—*Continued*

3,6-Endoxo-3-methyl-$\Delta^4$-tetrahydrophthalic anhydride
4-Ethoxy-3-nitrobenzoic acid
4-Ethoxyphenoxyacetic acid
N-Ethoxyphenyl-N′-phenylurea
Ethyl 4-bromophenoxyacetate
Ethyl 4-bromophenylcarbonate
Ethyl 4-chlorophenoxyacetate
Ethyl N-(3,5-dibromophenyl)-carbamate
Ethyl 2,4-dichlorophenoxyacetate
Ethyl 2,4-dichlorophenylcarbonate
Ethylene dicarbamate
3-Ethyl-5-methylphenoxyacetic acid
Ethyl pentachlorophenoxyacetate
2-Ethylphenoxyacetic acid
3-Ethylphenoxyacetic acid
4-Ethylphenoxyacetic acid
Ethyl N-phenylaminoacetate
Ethyl 2,4,5-trichlorophenoxyacetate
2-Fluorobenzoic acid
3-Fluorobenzoic acid
β-Fluoroethyl 2,4-dichlorophenoxyacetate
4-Fluoro-2-methylphenoxyacetic acid
2-Fluoro-3-nitrobenzoic acid
4-Fluorophenoxyacetic acid
2-Formylphenoxyacetic acid
4-Formylphenoxyacetic acid
Furoic acid
2-Furylacrylic acid
Glyceryl mono-2,4-dichlorophenoxyacetate
Hexamethylene dicarbamate
Hydrocinnamic acid
Hydrogen peroxide
Hydroquinone
2-Hydroxybenzoic acid
3-Hydroxybenzoic acid
4-Hydroxybenzoic acid
β-Hydroxyethyl 4-chlorophenoxyacetate
β-Hydroxyethyl 2,4-dichlorophenoxyacetate
N-(β-Hydroxyethyl)-α-naphthylamine
3-Hydroxyjuglone
5-Hydroxy-1,4-naphthaquinone (juglone)
2-Hydroxy-3-methoxybenzaldehyde
2-Hydroxy-3-nitrobenzoic acid
2-Hydroxy-5-nitrobenzoic acid
4-Hydroxyphenoxyacetic acid
4-Hydroxyphenylacetic acid
4-Hydroxyphenylglycine
4-Hydroxyphenylpyruvic acid
α-(*o*-Hydroxyphenyl)-α-toluic acid
3-Hydroxy-7-sulfo-2-naphthoic acid
3-α-Iminoethyl-5-methyltetronic acid
3-Indoleacetic acid
Inosityl hexa-(2,4-dichlorophenoxy)-acetate
2-Iodobenzoic acid
3-Iodobenzoic acid
4-Iodobenzoic acid
4-Iodophenoxyacetic acid
4-Iodophenylacetic acid
Isobutyl 4-chlorophenoxyacetate
α-Isonitrosoethylphenylketone
Isopropyl 4-chloro-2-methylphenoxyacetate
Isopropyl 4-chlorophenoxyacetate
Isopropyl N-(2-chlorophenyl)-carbamate
Isopropyl N-(3,5-dibromophenyl)-carbamate
2-Isopropyl-5-methylphenoxyacetic acid
Isopropyl N-phenylcarbamate
Isopropyl 2,4,5-trichlorophenoxyacetate
Lauroyl peroxide
Malonic acid
Mandelic acid
4-Menthane hydroperoxide
β-Methallyldiphenylacetic acid
2-Methoxycinnamic acid
2-Methoxy-4-methylphenoxyaceto-2′,5′-dichloroanilide
4-Methoxy-3-nitrobenzoic acid
4-Methoxyphenol
2-Methoxyphenoxyacetic acid
α-(2-Methoxyphenoxy)-*n*-butyric acid
3-Methoxyphenylacetic acid
2-Methylbenzoic acid
3-Methylbenzoic acid
Methyl 4-chlorophenoxyacetate
Methyl 2,4-dibromophenoxyacetate
Methyl N-(3,5-dibromophenyl)-carbamate
Methyl 2,4-dichlorophenoxyacetate
Methyl β-(furfuryloxy)-propionate
5-Methyl-2-nitrobenzoic acid
2-Methylphenoxyacetic acid
3-Methylphenoxyacetic acid
4-Methylphenoxyacetic acid
α-(2-Methylphenoxy)-*n*-butyric acid
α-(3-Methylphenoxy)-*n*-butyric acid
α-(4-Methylphenoxy)-*n*-butyric acid
2-(3′-Methylphenoxy)-ethanol
α-(2-Methylphenoxy)-propionic acid
α-(3-Methylphenoxy)-propionic acid
N-Methyl-N-phenylacetamide
3-Methyl-1-phenyl-5-pyrazolone
Methyl 2,3,5-tribromobenzoate
Methyl 2,4,5-trichlorophenoxyacetate
2-Methyl-4,x,y-trichlorophenoxyacetic acid
β-Methylumbelliferone
2-Naphthaleneacetic acid
1,8-Naphthalenedicarboxylic acid
Naphthalene-β-sulfonic acid monohydrate
2-Naphthoxyacetamide
1-Naphthoxyacetic acid
1-Naphthylacetohydrazide
1-Naphthylnitromethane

TABLE 3—*Continued*

Nicotinamide
Nicotinic acid
4-Nitro-9(10H)-acridone
3-Nitrobenzoic acid
4-Nitrobenzoic acid
3-Nitrocinnamic acid
4-Nitrocinnamic acid
2-Nitrophenol
2-Nitrophenoxyacetic acid
3-Nitrophenoxyacetic acid
4-Nitrophenoxyacetic acid
2-Nitrophenylacetic acid
4-Nitrophenylacetic acid
Octylphenoxydiethoxyethanol
Octylphenoxyethanol
Octylphenoxytetraethoxyethanol
2-Oximinomethylphenoxyacetic acid
4-Oximinomethylphenoxyacetic acid
Pentachlorophenoxyacetic acid
Peracetic acid
2-Phenanthreneacetic acid
3-Phenanthreneacetic acid
Phenoxyacetic acid
α-Phenoxybutyric acid
γ-Phenoxybutyric acid
2-Phenoxyethanol
β-Phenoxyethylmalonic acid
Phenoxyisobutyric acid
2-Phenoxypropionic acid
3-Phenoxypropionic acid
Phenylacetic acid
2-Phenylbutyric acid
N-Phenylglycine
N-Phenyl-N′,N′-(pentamethylene)-urea
4-Phenylureidobenzoic acid
*o*-Phthalic acid
*m*-Phthalic acid
*p*-Phthalic acid
Pimelic acid
Potassium *cis*-5-chloro-2-hydroxycinnamate
Proline
Propyl 4-chloro-2-methylphenoxyacetate
Propyl 4-chlorophenoxyacetate
4-Propylphenoxyacetic acid
4-Pyridinecarboxylic acid
Quercitin
6-Quinolinecarboxylic acid
Quinone
Rutin
Salicylaldoxime
5 copoletin
Sodium anthraquinone-β-sulfonate
Sodium azide
Sodium benzenesulfinate
Sodium β-(2,4-dichlorophenoxy)-ethyl-sulfate
Sodium β-naphthoquinone-4-sulfonate
Sodium *p*-toluenesulfinate
Sorbic acid
Succinonitrile
Sulfamerazine
Sulfanilamide
Syringic acid
Tetrachlorohydroquinone
Tetrachloroquinone
Thiodiphenylamine
2-Thiophenecarboxylic acid
2-Tolylurea
Traumatic acid
2,4,6-Tribromobenzonitrile
2,4,5-Trichloroacetanilide
Trichloracetic acid
2,4,5-Trichloroanisole
1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)-ethane
1,1,1-Trichloro-2,2-bis-(*p*-fluorophenyl)-ethane
1,1,1-Trichloro-2,2-bis-(*p*-methoxyphenyl)-ethane
2,4,5-Trichlorophenoxyacetamide
2-(2′,4′,5′-Trichlorophenoxyacetamide)-2-(hydroxymethyl)-1,3-propanediol
2,4,5-Trichlorophenoxyacetanilide
2,3,4-Trichlorophenoxyacetic acid
2,3,6-Trichlorophenoxyacetic acid
2,4,5-Trichlorophenoxyacetic acid
2,4,6-Trichlorophenoxyacetic acid
3,4,5-Trichlorophenoxyacetic acid
2,4,5-Trichlorophenoxyaceto-4′-chloroanilide
α-(2,4,5-Trichlorophenoxy)-*n*-butyric acid
γ-(2,4,5-Trichlorophenoxy)-*n*-butyric acid
α-(2,4,6-Trichlorophenoxy)-*n*-butyric acid
2-(2′,4′,5′-Trichlorophenoxy)-ethanol
2-(2′,4′,5′-Trichlorophenoxy)-ethyldiethylamine
α-(2,4,5-Trichlorophenoxy)-propionic acid
β-(2,4,5-Trichlorophenoxy)-propionic acid
α-(2,4,6-Trichlorophenoxy)-propionic acid
3-Trifluoromethylphenoxyacetic acid
3,4,5-Trihydroxybenzoic acid
Trimesic acid
3,4,5-Trimethoxybenzoic acid
2,4,5-Trimethylphenoxyacetic acid
3,4,5-Trimethylphenoxyacetic acid
α-(2,4,5-Trimethylphenoxy)-*n*-butyric acid
α-(3,4,5-Trimethylphenoxy)-propionic acid
4-(Trimethylsilyl)-phenylacetic acid
Tryptophane
4-Xenyloxyacetic acid

exhibited the same order of effectiveness as IAA, whereas 1-naphthalene-acetic acid was appreciably more potent. No antagonism was shown by phenylacetic acid or phenoxyacetic acid. It seems not unlikely that compounds, such as IAA, which may be rapidly transformed in the plant would have to be applied in considerably greater excess over the amounts actually participating in the competitive reaction than would compounds not subject to such inactivation.

Antagonism between TIBA and auxins has been demonstrated also in cell elongation and in flowering responses (**1, 2, 4, 6, 15**).

It is of some interest that tryptamine, a possible precursor of IAA in the plant, exhibits antagonistic activity to TIBA, albeit to considerably lower degree than does IAA itself. Possibly the low effectiveness may be due to tryptamine being too slowly converted to IAA to offset fully the inhibitory action of TIBA applied concurrently (**14**). Regardless of the interpretation of this result with respect to the locus of action of TIBA in the reaction chain, the antagonistic activity of tryptamine would seem to provide some additional evidence, admittedly of a circumstantial nature, that this compound may serve as a precursor of IAA.

Tryptophane, which may be regarded as the precursor of tryptamine, did not show antagonism to TIBA when tested at levels comparable with those of tryptamine. It should be pointed out, however, that the low solubility of tryptophane sets a limit to the amount that can be introduced into the bud by the present technique.


## Summary

1. Excision of the terminal bud of the seedling of *Phaseolus vulgaris* var. Black Valentine results in the formation of an abscission layer in the subjacent internode. A similar result can be induced by application of various chemicals to the intact bud.


TABLE 4

ANTAGONISM OF ABSCISSION-INDUCING ACTION OF 2,3,5-TRIIODOBENZOIC ACID BY 3-INDOLEACETIC ACID. COMPOUNDS APPLIED IN ADMIXTURE. PLANTS CULTURED IN ARTIFICIAL LIGHT. EXPERIMENT 660

| Dose TIBA applied (μgm.) | Dose IAA applied (μgm.) | Moles IAA/ Moles TIBA | Percentage of abscission |
|---|---|---|---|
| 0.05 | 0.0 | ..... | 80.0 |
| .05 | .10 | 5.7 | 6.7 |
| .05 | .15 | 8.6 | 0.0 |
| .05 | .20 | 11.4 | 0.0 |
| .1 | .0 | ..... | 100.0 |
| .1 | .2 | 5.7 | 6.7 |
| .1 | .3 | 8.6 | 0.0 |
| .1 | .4 | 11.4 | 0.0 |
| .2 | .0 | ..... | 100.0 |
| .2 | .4 | 5.7 | 28.6 |
| .2 | .6 | 8.6 | 8.3 |
| 0.2 | 0.8 | 11.4 | 0.0 |


2. The most effective compounds found, in tests of more than six hundred substances, were certain 2,3,5-trihalogenated benzoic acids; a large number of other compounds of considerable structural diversity also exhibit activity.

3. The abscission-inducing activity of 2,3,5-triiodobenzoic acid (TIBA) can be counteracted by concurrent application of a number of compounds including 3-indoleacetic acid and 1-naphthalenacetic acid.



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# EFFECT OF APPLICATIONS OF GROWTH SUBSTANCES ON SPROUTING OF CARROTS, TURNIPS, AND ONIONS IN STORAGE[1]

STEWART DALLYN AND ORA SMITH

## Introduction

SMITH and co-workers (**2**, **3**, **4**) applied solutions of sprout-inhibiting substances to the foliage of potatoes and found subsequent reduction in sprouting of the tubers during storage. In the present studies the work has been extended to include other crops. In the spring of 1948 several experiments were set up to determine the value of such applications on foliage of onions and carrots. These crops were selected because each represents a different form of storage organ and because both plants are important in the agricultural economy of New York.

In addition to the foliage treatments, applications were also made to the storage organs. Some information had been obtained previously, with successful results on carrots but not for onions. Accordingly, it was thought desirable to test several new materials which had become available and to obtain more information on rate and time of application of the commercially used inhibitors.

SMITH *et al.* (**4**) reported that tubers from plants sprayed with an emulsion of the methyl ester of naphthaleneacetic acid (MENA) sprouted much less than untreated control samples during storage. ELLISON and SMITH (**3**) found that most effective sprout inhibition was associated with early (July 17) application to the foliage.

[1] Paper no. 348, Department of Vegetable Crops, Cornell University, Ithaca, New York.

SMITH *et al.* (5) found that the sodium salt of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was much more effective in sprout retardation than sodium naphthaleneacetate (sodium NA). ELLISON (2) concluded that the most promising possibilities for obtaining sprout inhibition without incurring injury to yield and quality of tubers lay in the early application of low concentrations of 2,4,5-T.

## Material and methods

Sodium alpha-naphthaleneacetate (sodium NA) and sodium 2,4,5-trichlorophenoxyacetate (2,4,5-T) were applied to Red Core Chantenay carrots and Early Yellow Globe onions growing in muck soil in the Elba, New York, area. A factorial experiment involving three dates of application—July 9 and 24 and August 8—and three concentrations of the materials—500, 1000, and 3000 p.p.m. of sodium NA and 25, 50, and 100 (150 on onions) p.p.m. of sodium 2,4,5-T—was used. Sprays were applied at the rate of 80 gallons per acre with a knapsack sprayer.

An observational trial was made with twenty additional materials. All were applied at the rate of 50 p.p.m., 80 gallons per acre, on July 24. No yield data were taken, but the performance of the carrots and onions during storage was studied. The chemicals used were as follows: penta-chlorophenoxyacetic acid, alpha-naphthaleneacetamide, phenylacetic acid, potassium naphthaleneacetate, *p*-chlorophenoxyacetic acid, indole-3-acetic acid, alpha-2-chlorophenoxypropionic acid, alpha-2,4-dichlorophenoxypropionic acid, 2,4,6-trichlorophenoxyacetic acid, alpha-2,4,5-trichlorophenoxy propionic acid, n-phenyl isopropyl carbamate, beta-indole-3-propionic acid, potassium indoleacetate, indolebutyric acid, trichlorophenylacetic acid, ammonium 2,4-dichlorophenoxyacetate, isopropyl ester of 2,4-dichlorophenoxyacetic acid, beta-naphthoxyacetic acid, o-chlorophenoxyacetic acid, and alpha-o-chlorophenoxypropionic acid.

In an additional experiment various applications were made directly to the storage organs at intervals following harvesting. Samples from all experiments were stored at 50° F.

## Results and discussion

FOLIAGE SPRAYS ON CARROTS.—Applications of both chemicals in the main experiment resulted in reduced yields. This

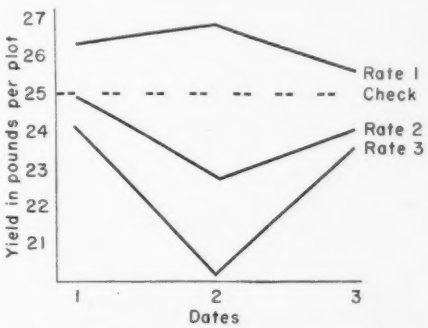


FIG. 1.—Effect of foliage treatments with sodium 2,4,5-T on yield of carrots (1 lb. = 0.5 ton per acre). Rate 1 = 25 p.p.m.; rate 2 = 50 p.p.m.; and rate 3 = 100 p.p.m. Treatment dates: 1 = July 9; 2 = July 24; and 3 = August 8.

reduction was accentuated both by the high rates and by early dates of application. The action of sodium 2,4,5-T (fig. 1) was much more severe than that of sodium NA, despite the fact that it was used in much lower concentrations.

Sodium NA treatments in general tended to reduce water loss during storage, though no single treatment was significantly different from the untreated. Sodium 2,4,5-T had little, if any, effect in this respect.

The general effect of both chemicals

TABLE 1

EFFECT OF GROWTH SUBSTANCES APPLIED AS FOLIAGE SPRAYS ON SPROUTING OF CARROTS IN STORAGE*

| CHEMICAL | CONCENTRATION P.P.M. | DATE OF APPLICATION | | |
|---|---|---|---|---|
| | | July 9 | July 24 | August 8 |
| Sodium NA | 500 | 28.9 | 15.3 | 18.4 |
| | 1000 | 28.4 | 20.4 | 30.3 |
| | 3000 | 22.5 | 12.8 | 23.1 |
| Sodium 2,4,5-T | 25 | 21.7 | 23.3 | 27.8 |
| | 50 | 16.6 | 11.5 | 25.6 |
| | 100 | 23.7 | 16.7 | 17.0 |
| Untreated control | 0 | 25.4 | | |

* Data (mean of four replications) given as grams of sprouts produced per 1100-gm. sample from September 7 to February 10.

was to reduce sprouting, but only two specific treatments resulted in statistically significant differences. These were 3000 p.p.m. sodium NA applied on July 24 and 50 p.p.m. sodium 2,4,5-T applied on the same date (table 1). Photographs of typical samples after storage at 50° F. for 115 days are included in figure 2.

Results of the experiment indicated that treatments which showed promise for sprout inhibition usually reduced crop yield. In an attempt to avoid this difficulty, high concentrations and high rates of application of these salts were sprayed on the plants late in the season after their growth had been largely com-

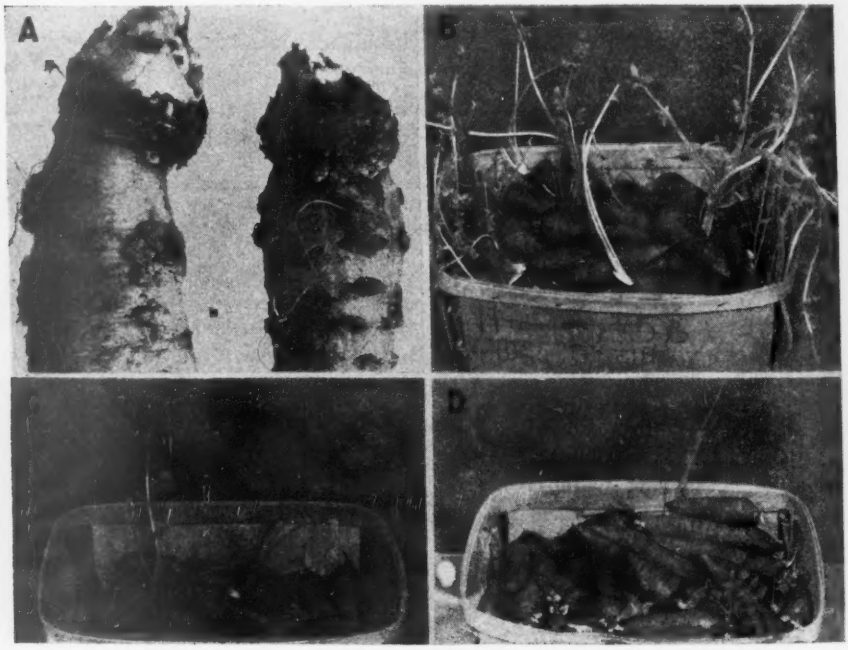


FIG. 2.—Effects of July 24 applications of chemicals to carrot foliage on roots in subsequent storage. *A*, most severely injured roots caused by 2,4,5-T, 100 p.p.m. *B*, untreated control after 115 days' storage at 50° F. *C*, storage sample from 2,4,5-T treatment, 100 p.p.m. *D*, storage sample from sodium NA treatment, 3000 p.p.m.

pleted. Sodium NA applied at 3000 p.p.m., 135 gallons per acre, on September 4 (8 days before harvesting) caused no root malformation or reduction in yield but resulted in a marked retardation of sprouting. Its use at that time appeared to be the most promising and is worthy of further investigation.

None of the twenty materials in the observational experiment induced any marked response under the conditions in which they were used.

APPLICATIONS DIRECTLY TO ROOTS OF CARROTS AND TURNIPS.—Three forms of 2,4,5-T—the sodium salt and the methyl and isopropyl esters—and methyl naphthaleneacetate (MENA), in dust carriers containing 2.2% of the active material, were applied directly to the roots of carrots and turnips. All materials effectively inhibited sprout growth (fig. 3). In general, however, the ester forms of 2,4,5-T were found likely to cause considerable root damage unless applied under fairly

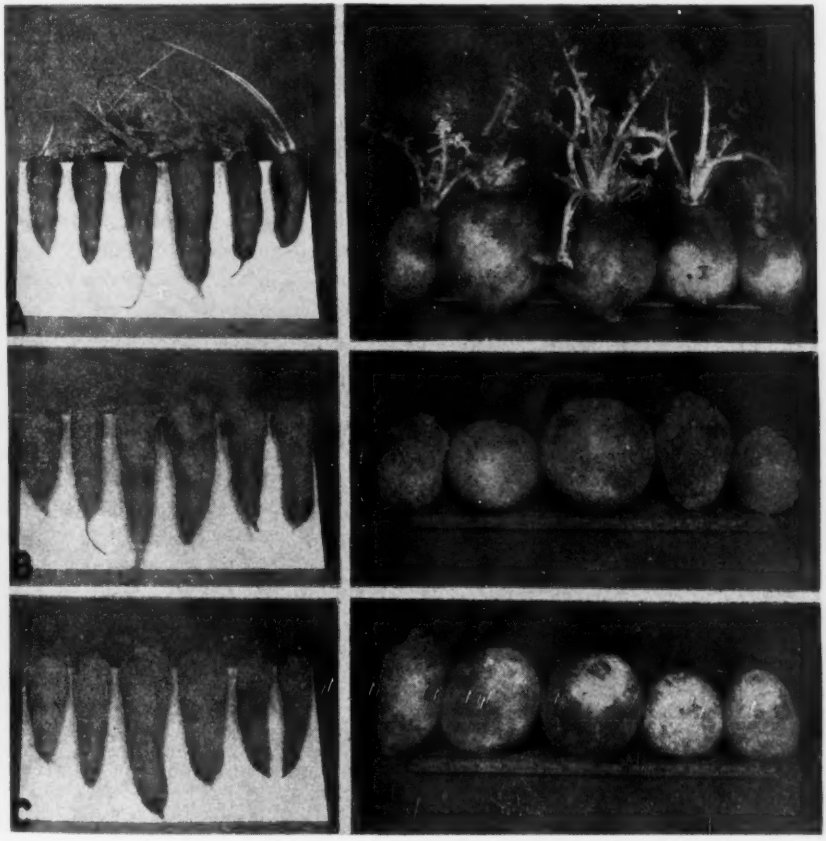

FIG. 3.—Appearance of carrots (*A*, *B*, *C*) and turnips (*D*, *E*, *F*) after storage for 115 days at 50° F. *A*, *D*, untreated controls. *B*, *E*, MENA applied at rate of 1 gm. (2.2% in dust carrier) per bushel, 4 weeks after roots had been placed in storage. *C*, *F*, methyl ester of 2,4,5-T used similarly to MENA.

specific conditions. Variation of time and amount of the materials used indicated that about 1 gm. of the pure chemical per bushel applied 4 weeks after the roots had been placed in storage was most satisfactory. Both the heavy and the six sodium NA treatments applied on the first two dates was higher at odds of more than 99:1 than those untreated. The chemical at 500 p.p.m. applied on July 9 induced a 19% increase in yield. These results appear sufficiently interesting to warrant further investigation of the growth substance in this role.

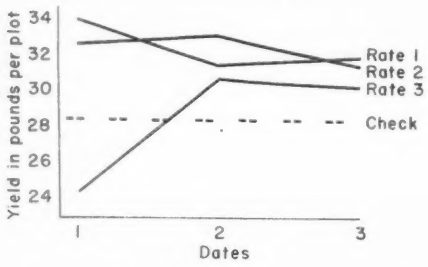


FIG. 4.—Effect of foliage treatments with sodium NA on yield of onions (1 lb. approximately equals 30 bushels per acre). Rate 1 = 500 p.p.m.; rate 2 = 1000 p.p.m.; and rate 3 = 3000 p.p.m. Treatment dates: 1 = July 9; 2 = July 24; and 3 = August 8.

The general trend in the effect of both chemicals was to reduce slightly the loss in weight during storage. High concentrations of the 2,4,5-T salt, however, caused a marked increase in weight loss.

During the 30-week storage period at 50° F. the samples were inspected at weekly intervals, and the number of sprouted bulbs was recorded. Several of the treatments had inhibitory effects on sprouting for the first half of the storage period, but these differences were not very marked at the end of 31 weeks of storage (fig. 5).

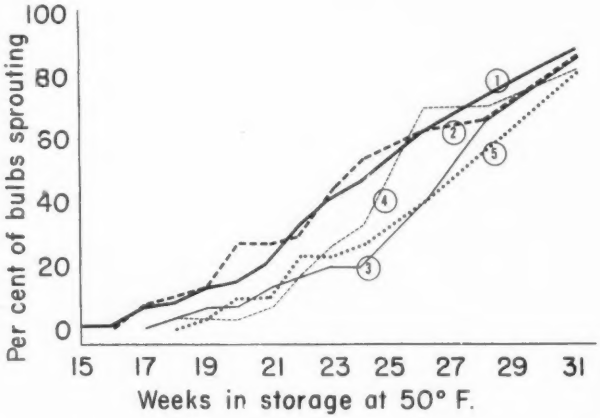


FIG. 5.—Effect of sodium naphthaleneacetate sprayed on onion plants on subsequent sprouting in storage: *1*, unsprayed; *2*, 1000 p.p.m. applied on July 9; *3*, 3000 p.p.m. applied on July 9; *4*, 1000 p.p.m. applied on August 8; *5*, 3000 p.p.m. applied on July 24.

early applications were somewhat detrimental to the roots.

FOLIAGE SPRAYS ON ONIONS.—The mean yield following sodium NA treatments (fig. 4) was higher than yields of the control or following application of sodium 2,4,5-T. The mean yield for the

In order to insure that the materials were getting inside the plants, the following treatment was made. On July 24 the tops of plants were cut off about a foot above ground level, and the sprays

were directed down into the open ends of the leaves. Bulbs from these plots sprouted as readily in storage as those from plots treated in the conventional manner.

None of the additional chemicals used in the exploratory experiment gave any indication of sprout suppression.

APPLICATIONS DIRECTLY TO ONION BULBS.—Treatments were the same as those used on the carrot and turnip roots. The three forms of 2,4,5-T caused weight loss of the bulbs in proportion to the activity of the chemical. Thus the methyl ester form brought about a highly significant increase in loss compared with the sodium salt. These effects were similar to those on carrots treated in the same way. MENA had no significant effect in this respect. None of the treatments had any influence on the incidence of rotting, which was extremely low in all onion experiments.

The materials used were of no benefit in controlling sprouting, nor did they have any noticeable effect on growth of the shoots after emergence. Onions would thus appear to be immune to the common growth-control practices. More recent experiments show that applications of maleic hydrazide suppress sprout growth.

Some preliminary work was carried out with toothpicks soaked in sodium NA and sodium 2,4,5-T. When these were inserted into dormant onions, the bulbs soon started to grow, while unpunctured control bulbs remained dormant for several weeks. Untreated toothpicks had the same effect as treated ones. This would indicate that some other factor than the applied chemical was involved, possibly availability of oxygen, as suggested by BOSWELL (1), or enzymes released by injury or other effects which follow wounding.

## Summary

1. Experiments with sodium naphthaleneacetate (sodium NA) and sodium 2,4,5-trichlorophenoxyacetate (sodium 2,4,5-T) applied as preharvest foliage sprays to carrots resulted in some sprout retardation in the harvested crop. The most promising results were obtained with light applications of sodium NA applied early in the season, or very heavy applications 8–10 days before harvest.

2. Three forms of 2,4,5-T (the sodium salt and the methyl and isopropyl esters) and methyl naphthaleneacetate (MENA), in dust carriers, were applied directly to harvested carrot and turnip roots. One gram of MENA per bushel of roots, applied 4 weeks after they had been placed in storage at 50° F., gave very satisfactory sprout inhibition.

3. Several different treatments with sodium NA applied to the foliage of onions reduced sprouting of the bulbs in subsequent storage at 50° F.

4. Low concentrations of sodium NA applied to the foliage of onions resulted in increased bulb yields.

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# TRANSLOCATION OF 2,4-DICHLORO-5-IODOPHENOXYACETIC ACID IN VELVET MESQUITE SEEDLINGS[1]

BYRON O. BLAIR[2] AND W. H. FULLER[3]

## Introduction

Erratic responses of velvet mesquite (*Prosopis juliflora* var. *velutina* [Woot.] Sarg.), a perennial sprouting leguminous tree, to spray applications of different organic herbicides to the foliage indicate a lack of absorption, translocation, or both. Quantitative measurements of the movement of 2,4-D derivatives appear to be essential to a satisfactory explanation of the resistance of some plants to these herbicides but could not be made until recently. During the past year MITCHELL and LINDER (2) reported the successful use of radioactive iodine ($I^{131}$) substituted in 2,4-D and several of its derivatives for making quantitative determinations of the absorption and translocation of this material in young bean seedlings. They found that the addition of adjuvants (co-solvents and surface-active agents) resulted in increased absorption of 2,4-dichloro-5-iodophenoxyacetic acid (2,4-$DI^{131}$) by young bean seedlings. This paper reports on techniques used and quantitative data obtained on translocation of 2,4-$DI^{131}$, with and without added adjuvants, applied at herbicidal concentrations as a spray to foliage of velvet mesquite seedlings.

## Material and methods

A radioactive morpholine salt of 2,4-$DI^{131}$ prepared by the Texas Research Foundation was used. The cost of material and the difficulty of handling a herbicidal concentration of active material necessitated the dilution of the radioactive material with nonradioactive 2,4-DI at a 1:12 ratio. Aqueous solutions were prepared by dissolving the diluted radioactive material in distilled water. Those containing adjuvants were prepared by adding 1% by volume of the adjuvant to the water before the herbicide was added. Adjuvants tested were Tween 20, Shell Weed Killer emulsifier, and Riverdale Dispersant no. 6. Concentration of the combined 2,4-DI and 2,4-$DI^{131}$ in all solutions was 850 p.p.m. on an acid-equivalent basis. One milliliter of solution was applied to each seedling. This amount was sufficient to wet the foliage but did not result in runoff.

Spraying was done with a DeVilbiss aqueous-solution atomizer modified to permit accurate administration of 1-ml. quantities. A length of glass tubing large enough to permit the siphon tube of the atomizer to be fitted into it was sealed at one end and set in wax in the center of the reservoir bottle. The glass siphon tube was then heated and drawn into a small capillary and cut at a length which would reach the bottom of the improvised inner reservoir. A DeVilbiss auto-

[1] This study is part of investigations being conducted by the Southwestern Forest and Range Experiment Station in co-operation with the University of Arizona Agricultural Experiment Station under RMA project RM:b-4 for research on the basic ecology and physiology in control of undesirable range plants.

[2] Range Conservationist, Southwestern Forest and Range Experiment Station, maintained by the Forest Service, U.S. Department of Agriculture for Arizona, New Mexico, and West Texas, with headquarters at Tucson, Arizona; [3] Associate Biochemist, Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona.

matic cutoff control valve was used in the air line. All spraying was done under 20 pounds pressure.

The test plants were grown from seed in no. 10 tin cans under optimum moisture conditions for 8 weeks prior to spraying. At the time of spraying the tops of plants selected for treatment averaged 13 inches in height. Of this only the upper 6 inches, comprising, on the average, six nodes and thirteen leaves, were sprayed. Five seedlings were sprayed with each solution tested.

The technique used for isolating the parts to be sprayed and preventing contamination of the laboratory was as follows: A wooden box, approximately 12 inches square and with a $2\frac{1}{2}$-inch hole in its top, was placed over the no. 10 can so that the top of the seedling extended through the hole. Four 6-inch filter papers, cut from edge to center, were carefully fitted around the stem and over the hole in the box in such a manner as to prevent spray material from reaching the lower portion of the seedling. A bell jar 6 inches in diameter and 9 inches tall with a 1-inch opening in the top was then placed on top of the box with the hole centered over the exposed portion of the seedling. The spray tip, fitted with a rubber stopper, was then inserted into the hole at the top of the bell jar. After each plant was sprayed, an interval of 1 minute was allowed before removing the bell jar. This interval was adequate to permit the atomized spray to settle within the jar and on the plant (fig. 1).

Four days (96 hours) after the activated herbicide was administered, the plants were removed from the cans and dissected. Tissue samples were taken from that part of the stem below the area sprayed and above the cotyledonary node, from the hypocotyl, and from the lower roots. Samples were dried and ground in a Wiley mill so as to pass a 40-mesh screen. Aliquot samples containing 25 mg. of this tissue were then prepared for analysis using Scotch tape and standard diameter brass rings, as described by MITCHELL and LINDER (3).

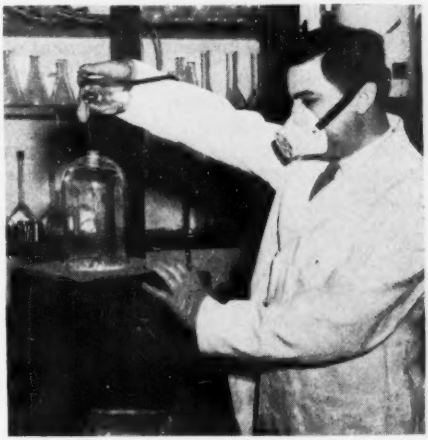

FIG. 1.—Technique used to spray isolated tops of mesquite seedlings under bell jar with radioactive 2,4-DI$^{131}$ administered into top of jar as 850-p.p.m. spray.

Activity of the samples was determined quantitatively at the counting laboratory of the University of Arizona.

## Results and discussion

The amount of herbicide which came in contact with each plant in each treatment was measured. Disintegration counts showed that, of the active material applied (361,000 d/s), an average of 4.32% was recovered on treated areas of the plants (table 1). The small variation among treatments is attributed to inherent differences in number, size, and orientation of leaves and to the impossibility of arranging each plant in the bell jar in identical relation to the spray tip. These data give an indication of the low

efficiency of foliage sprays on sparsely foliated plants and may indicate the need for high-volume applications on species which have so far been difficult to kill.

Downward movement of herbicide from treated top segments into untreated portions was limited. In all cases over 97% of the material which was deposited on the foliage remained in the treated area at the end of 96 hours. Movement of 2,4-DI$^{131}$ in water (treatment A) from the treated area into untreated areas was inversely proportional to distance—1.21, 0.60, and 0.45% in stems, hypocotyls, and roots, respectively. Although total movement of 2,4-DI$^{131}$ in solutions containing adjuvants was less than that in water alone, it is of interest that a greater concentration of 2,4-DI$^{131}$ was found in the root than in hypocotyl or untreated stem tissue when solutions contained Shell or Riverdale emulsifiers. The differences are small, in view of the very limited amounts of herbicide which moved out of the treated area in each of these two treatments, but may indicate that movement to roots was enhanced by the adjuvants.

The addition of adjuvants increased the toxicity of the herbicide to the tissue that was sprayed. Examination of treated areas showed extensive damage to leaves and stems in treatments B and D, slightly less damage to C, and only a temporary change in leaf orientation in treatment A (fig. 2).

All plant tissue treated with 2,4-DI$^{131}$ in aqueous solutions containing adjuvants was dead in 10 days. That treated with 2,4-DI$^{131}$ in water alone appeared undamaged 6 weeks after application. It has been reported that 2,4-DI$^{131}$ is only about 60% as toxic as 2,4-D, and that toxicity to tissue treated can be increased many fold by adding an adjuvant (1). In general, data in the present study show that increased toxicity to the area treated may bear an inverse relationship to the amount of translocation of the herbicide from that area. As compared with that in treatment C, top kill in B and D was rapid; this may account for the small amount of movement of 2,4-DI$^{131}$ downward from the treated area in these two treatments.

It is believed that the increased local toxicity noted in B, C, and D, compared with A, resulted from increased absorp-

TABLE 1

AVERAGE PERCENTAGE MOVEMENT OF ACTIVE MATERIAL FROM TREATED AREA OF STEM TO UNTREATED STEM, HYPOCOTYL, AND ROOT TISSUE OF VELVET MESQUITE SEEDLINGS 96 HOURS AFTER APPLICATION

| Treatment | Carrier for 2,4-DI$^{131}$ | Total I$^{131}$ recovered (%) | Distribution in plant tissue (%) | | | |
|---|---|---|---|---|---|---|
| | | | Treated area | Stem | Hypocotyl | Root |
| A | Water | 4.42 | 97.74 | 1.21 | 0.60 | 0.45 |
| B | Water+1% Tween 20 | 4.63 | 99.35 | 0.33 | .18 | .14 |
| C | Water+1% Shell Weed Killer Emulsifier | 3.74 | 98.89 | 0.21 | .21 | .69 |
| D | Water+1% Riverdale no. 6 | 4.50 | 99.52 | 0.16 | 0.10 | 0.22 |

tion of 2,4-DI$^{131}$. It may well be that, where 2,4-D compounds are used for controlling annual weeds or nonsprouting perennials, the use of an adjuvant would be desirable.

## Summary

1. The movement of the radioactive herbicide 2,4-dichloro-5-iodophenoxyacetic acid (2,4-DI$^{131}$) from treated tops to untreated lower stem, hypocotyl, and root tissue in 8-week-old velvet mesquite seedlings was determined quantitatively. A simple technique by which the upper half of the seedling tops was isolated for spraying is described.

2. Direct comparisons of the amount of movement of 2,4-DI$^{131}$ applied in water and in aqueous solutions to which three different surface-active agents were added as adjuvants were obtained.

3. Results obtained from Geiger-count analyses show that 96 hours after spraying less than 3% of the deposited radioactive material had moved from the treated seedling tops to tissue located more basally.

4. The addition of adjuvants at a rate of 1% by volume did not result in greater total translocation of the herbicide from the treated area but substantially increased the degree of acute local toxicity on the tissue sprayed—probably as a result of increased absorption. Addition of adjuvants also resulted in translocation of greater relative amounts of the herbicide to root and hypocotyl than to the stem.

5. The extremely limited movement of 2,4-DI$^{131}$, as measured in this study, may indicate why 2,4-D and other phenoxy compounds have given erratic results when applied as herbicides on mature velvet mesquite trees.

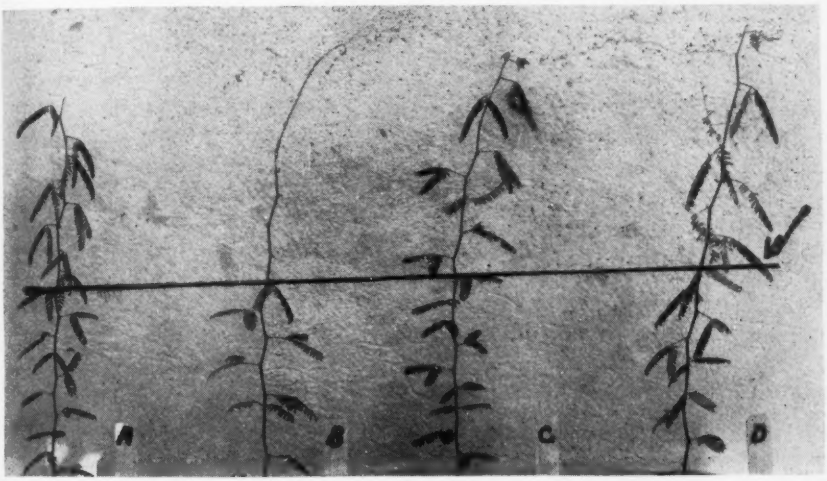


FIG. 2.—Typical seedlings photographed 10 days after application of 850-p.p.m. 2,4-DI$^{131}$ in water. Only that part of plant above line indicated by arrow was sprayed. In *B*, *C*, and *D*, respectively, 1% of Tween 20, Shell Weed Killer emulsifier, and Riverdale Dispersant no. 6 was added. Note complete defoliation in *B* and partial defoliation and shriveling of leaves in *D* and *C* as compared with temporary abnormal leaf position only in treatment *A*.

## LITERATURE CITED

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